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A STUDY OF THE COTTON FLEA HOPPER, PSALLUS SERIATUS REUT., WITH ESPECIAL REFERENCE TO ITS EFFECT ON COTTON PLANT TISSUES[1] [2]

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INTRODUCTION

The cotton flea hopper, *Psallus seriatus* Reuter, which belongs to the family Miridae, has only recently become important as a cotton pest (*5*).[4] The spread through the Southern States of a peculiar form of injury traceable to this insect has been rapid, although the insect itself has been known to be present there for some time. The generalized effect of the attack of the insect on the cotton plant leads one to look for the presence of other factors in addition to simple insect feeding.

During the summer of 1925 an investigation was made of the possibility of the transmission of a plant disease by the cotton flea hopper, and in order to get a definite anatomical basis for future work both with regard to the insect and the injured plant structure the study was made in three parts: (1) A study of the anatomy of the digestive apparatus of the insect; (2) a study of pathological and of healthy plant tissue; and (3) an examination of the fresh material for verification of findings in preserved material and examination of digestive enzymes present.

TECHNIC

The material intended for preservation and later study was collected at Port Lavaca, Tex., and after fixatiou was preserved in 80 per cent alcohol until used at Columbus, Ohio. Eight different fixing fluids were used. Bouin's, Zenker's, and Petrunkewitsch's fixing fluids were found to be the best for insect material. Bouin's fluid and a formalin-acetic acid-alcohol formula by Chamberlain (*2*) were found to be the best for the plant material. Heidenhain's iron haematoxylin, used by the longer method, and Ehrlich's acid haematoxylin, both counterstained with orange G., were found to be the most satisfactory stains for both plant and insect tissues.

[1] Received for publication July 24, 1929; issued March, 1930.

[2] This paper was presented in August, 1926, as a dissertation in partial fulfillment of the requirements for the degree of doctor of philosophy in the graduate school of the Ohio State University. The work upon which it is based was begun while the author was in the status of temporary field assistant in the Bureau of Entomology, U. S. Department of Agriculture, and completed in 1926 while he was in the status of collaborator of the Bureau of Entomology and graduate student, Ohio State University.

[3] The first experiments were made under the direction of the late Dr. W. D. Hunter, to whom the writer is indebted for many useful suggestions. The writer wishes to thank the members of the faculty of the department of zoology and entomology of Ohio State University, especially Drs. Herbert Osborn, Raymond C. Osburn, and C. H. Kennedy, and Dr. H. C. Sampson, of the department of botany, for valuable assistance and suggestions. Thanks are also due to members of the U. S. Bureau of Entomology, especially to Mr. B. R. Coad and Dr. J. W. Folsom, for making possible the investigation and for the excellent facilities furnished at the Delta laboratory, Tallulah, La., during July, 1926. The writer wishes also to thank Mr. Lawrence Youman, of Columbus, Ohio, for help with the photomicrographs of plant tissue. Most of the microtechnic work in connection with the preparation of slides for study was done by Mrs. R. H. Painter, to whose efforts was due the excellence of the material that was studied. This manuscript was submitted to the Bureau of Entomology for publication Sept. 28, 1926.

[4] Reference is made by number (italic) to "Literature cited," p. 516.

In studying the gross anatomy of these minute insects the following method was used for dissection and found to be successful if a strong enough illumination was used: The insect which had been fixed and stored was removed from the alcohol and allowed to dry a few minutes on filter paper. It was then placed on its side in a small drop of melted paraffin on the bottom of a watch-glass dissecting dish and completely covered with a thin coat of paraffin. The dish was flooded with cold 50 per cent alcohol and placed under the binocular. Scalpels were prepared by cementing a scrap of safety-razor blade in the end of a glass tube. With these it was possible to remove the exoskeleton together with the paraffin and expose the internal organs. The alcohol was then poured off. A drop of Ehrlich's acid haematoxylin was placed on the insect and allowed to stand for about five minutes and was then washed off. Dissection was then resumed under alcohol. This method does not stain entirely through the insect at one time, and it is often necessary to repeat the staining process. The coloring of the insect, a part at a time, is often of advantage. Organs may be stained in this manner and removed for mounting on slides.

Although a number of other hemipterous forms have been studied with regard to their internal anatomy and histology, relatively little is known about the Miridae in this respect.

Dufour (*3*), in 1833, figured and described the anatomy of the digestive tract of two European mirids, but the writer has been unable to find a description of the histology of any member of the family.

STRUCTURE AND HISTOLOGY OF THE ALIMENTARY CANAL AND ITS APPENDAGES

The structure of the mouth parts and salivary apparatus of *Psallus seriatus* is, on the whole, similar to that of other Hemiptera. With regard to homologies of parts the writer has followed the terminology [5] given in a General Textbook of Entomology, by Imms (*6*), and no attempt has been made to study these parts in regard to their relationship to similar parts in other members of the order. Hence the parts will be only briefly described and any structures peculiar to this insect will be noted. The description below applies to the adult except as otherwise mentioned.

MOUTH PARTS

The labium is 4-segmented and is furnished at the tip, as is usual in the order, with sensory hairs and pits. The proximal segment is quite heavily muscled for the bending of this organ at the time of feeding. This musculature, however, has not been worked out in detail. A groove, which is not entirely closed at any point, runs the length of this rostrum, except at its extreme basal end, and contains the stylets. Above the basal end of these mouth parts are the epipharynx and labrum, which are not easily distinguishable from each other. At the base of these is a groove in which the stylets lie.

The stylets consist of the mandibles and maxillae, all of which are hollow except at the extreme tips, and at their bases possess glands and muscular attachments. In the head each pair of stylets

[5] A paper by Snodgrass (*9*), which appeared after this manuscript was submitted for publication, gives a different and probably better interpretation of the parts of the hemipteran head. An explanation of the function of the "retort-shaped organs" described herein is also given in his paper.

is somewhat enlarged and flattened, the mandibles lying above the maxillae. As they pass into the groove of the epipharynx, the mandibles take up a position on each side of the maxillae. The latter possess two grooves on the inner faces which, when opposed to one another, form dorsally the suction canal and ventrally the ejection canal. The former connects with the pharynx, the latter with the salivary duct, opening through the hypopharynx. The stylets themselves are interlocked and grooved (fig. 1, D) and are furnished with ridges which fit into grooves in the ventral plate of the epipharynx where the stylets emerge from the head. At their tips the mandibles are of a heavier structure and are slightly shorter than the maxillae, and, whereas the latter are thin and barbed on their dorsal edge, the former are apparently smooth.

In the attachment of the stylets there are apparently no levers such as are present in some of the other Hemiptera, but each appendage is attached by a pair of muscles, as shown in Figure 1, E and F. In the head the upper and inner pair of stylets are the mandibles. The small dorsal muscle of the mandible attaches to the vertex after passing out just in front of the optic nerve. The ventral muscle passes beneath and to the side of the brain and attaches to the occiput or back wall of the head capsule. (Fig. 1, E.) On the maxillae the muscles are larger; one passes anteriorly, attaching to the maxillary sclerite near its junction with the labrum; the other extends posteriorly, passing beneath and behind the optic nerve and attaching to the occiput just below and to the side of the origin of the ventral mandibular muscle. (Fig. 1, F.)

There is a gland at the base of each stylet which in the adult is small. At the base of the maxilla it is usually L-shaped, extending laterally and dorsally, with the expanded bladelike portion of the stylet forming a chitinous wall to part of the gland; at the base of the mandible it is more pyriform, lying above, and external to, the hollow chitinous rod. The cavities of these glands are continuous with the cavities of the stylets and sometimes show tissue inside the base of the cavities. These glands are apparently the homologues of the "retorten formigen Organen" of German authors.

In the third, fourth, and fifth nymphal stages the stylets are each connected with spiral coils of tissue lying in the prothorax, which are much more like the "retort-shaped organs" of aphids and other Homoptera. These coils and the connections with the stylets are hollow and are lined with a chitinous membrane except at the proximal end in the interior of the coil. This enlarged proximal end and some of the tissue surrounding the coil consist of a compact mass of quite distinct cells with enlarged nuclei. Toward the end of the fifth nymphal stage these structures degenerate entirely, and the glands at the base of the stylets in the adult are all that are left. The details of these structures are not included in this paper, as they apparently have no intimate relation to the digestive secretions of the insect.

BUCCAL GLANDS

Immediately above the place where the maxillae come together to form the suction and ejection canals lie certain interesting cells which may perhaps be best termed the "buccal gland cells." In this region, which probably represents all that is left of the mouth cavity in the Hemiptera, the dorsal plate, a continuation of the dorsal

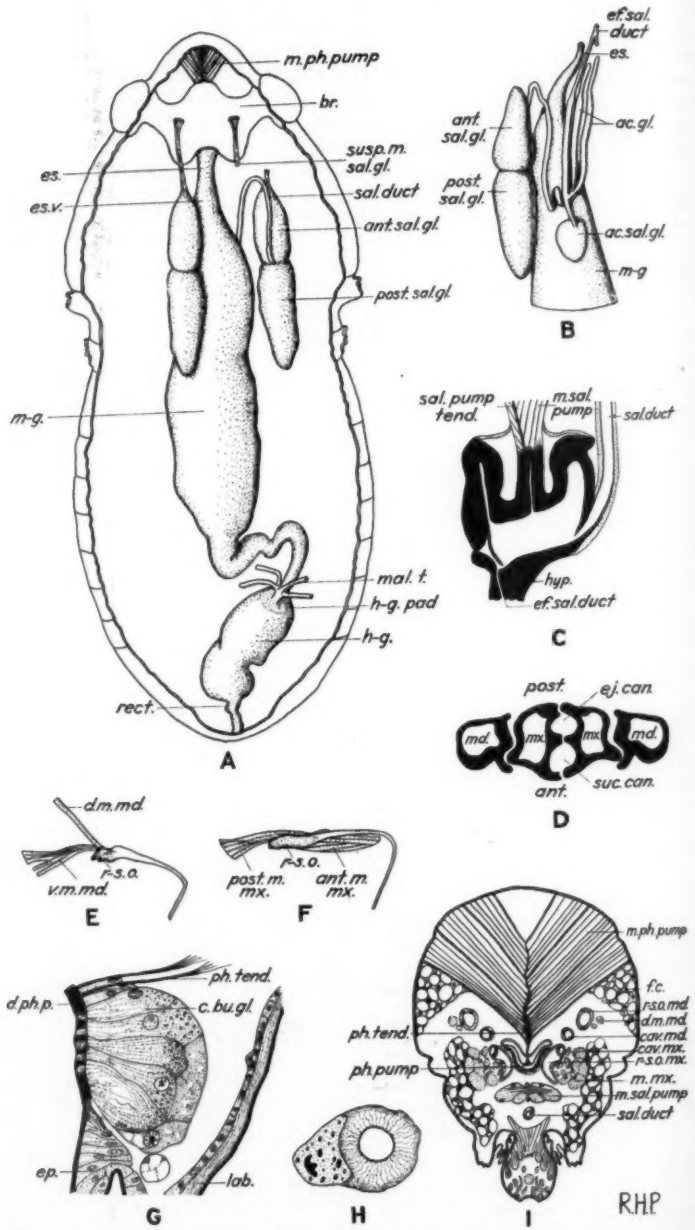

FIGURE 1.—The cotton flea hopper, *Psallus seriatus*. (For explanatory legend see opposite page)

pharyngeal plate, is perforated by two longitudinal rows of five pores each, above which lie the gland cells. A longitudinal section of these structures is shown in Figure 1, G. Near the pores the cytoplasm of these cells is sparse and fibrous; the distal ends of the cells, in which lie the nuclei, are filled with large granules that take haematoxylin stain quite deeply. Several cells containing cytoplasm of quite a different structure lie beyond the distal end of the gland cells proper.

The function of these glands is problematical, and probably they are too small for experimentation. Their secretion, which is of small quantity, mingles with the incoming food and may in some way take the place of the secretion of the salivary glands which, in other insects, open at about this point, since in this insect the salivary glands proper do not communicate directly with the alimentary canal. The buccal gland cells may be sensory in function, as for taste, but the writer has been unable to find a nervous connection of any size; moreover, these cells have the appearance of having the function of secretion.

ALIMENTARY CANAL

For the purpose of description the alimentary canal may be divided conveniently into the fore-gut consisting of the pharynx and the esophagus, the undifferentiated mid-gut separated from it by the esophageal valve, and the hind-gut. Like the mid-gut, the hind-gut shows little or no separation into distinct divisions.

The pharynx extends from the buccal glands back to a point almost beneath the center of the brain. In cross section it is horseshoe shaped and consists of a heavy chitinous ventral plate connected with a lighter dorsal plate by a region thinly chitinized. Surrounding this structure is a very thin layer of small epithelial cells; few or no muscular elements are to be seen, either as circular or longitudinal muscles. Piercing this epithelial layer and attached to the center of the dorsal plate is a comblike structure of erect chitinous tendons. This structure runs the entire length of the pharynx. From their point of attachment on the chitinous tendons, divergent muscles extend to the sides and are fastened to the vertex and front, appearing V-shaped

EXPLANATORY LEGEND FOR FIGURE 1

A.—General anatomy of the alimentary canal and salivary glands, drawn from dissection and sections × 107; *ant. sal. gl.*, anterior salivary gland; *br.*, brain; *es. v.*, esophageal valve; *es.*, esophagus; *h-g.*, hind-gut *h.-g. pad*, hind-gut pad; *m-g.*, mid-gut; *m. ph. pump*, muscles of salivary pump; *mal. t.*, Malpighian tubule *post. sal. gl.*, posterior salivary gland; *rect.*, rectum; *sal. duct*, salivary duct; *susp. m. sal. gl.*, suspensory muscle of salivary gland.

B.—Salivary gland, duct, and accessory gland, drawn from a dissection of a last-stage nymph, × 107; *ac. gl.*, accessory gland; *ac. sal. gl.*, accessory salivary gland; *ant. sal. gl.*, anterior salivary gland; *ef. sal. duct*, efferent salivary duct; *es.*, esophagus; *m-g.*, mid-gut; *post. sal. gl.*, posterior salivary gland.

C.—Longitudinal section of the salivary pump, drawn from an unstained whole mount, × 893; *ef. sal. duct*, efferent salivary duct; *hyp.*, hypopharynx; *m. sal. pump*, muscles of salivary pump; *sal. duct*, salivary duct; *sal. pump tend.*, salivary pump tendon.

D.—Cross section of stylets just after they emerge from the head, × 893; *ant.*, anterior; *ej. can.*, ejection canal; *md.*, mandible; *mx.*, maxillae; *post.*, posterior; *suc. can.*, suction canal.

E.—Base of mandible with retort-shaped organ and muscular attachments, × 481; *d. m. md.*, dorsal muscle of mandible; *r-s. o.*, retort-shaped organ; *v. m. md.*, ventral muscle of the mandible.

F.—Base of maxillae with retort-shaped organ and muscular attachments, × 481; *ant. m. mx.*, anterior muscle of maxillae; *post. m. mx.*, posterior muscle of maxillae; *r-s.o.*, retort-shaped organ.

G.—Longitudinal section of buccal gland cells and pores, × 893; *c. bu. gl.*, cells of buccal gland; *d. ph. p.*, dorsal pharyngeal plate; *ep.*, epipharynx; *lab.*, labrum; *ph. tend.*, pharyngeal tendon.

H.—Cross section of salivary duct, × 413.

I.—Cross section of head just in front of the eyes, × 308; *cav. md.*, cavity of mandible; *cav. mx.*, cavity of maxillae; *d. m. md.*, dorsal muscle of mandible; *f. c.*, fat cells; *m. mx.*, muscle of maxillae; *m. ph. pump*, muscles of pharyngeal pump; *m. sal. pump*, muscles of salivary pump; *ph. pump*, pharyngeal pump; *ph. tend.*, pharyngeal tendon; *r-s. o. md.*, retort-shaped organ of mandible; *r-s. o. mx.*, retort-shaped organ of maxillae; *sal. duct*, salivary duct.

in cross section. (Fig. 1, A and I.) The entire structure may be called the pharyngeal pump.

The esophagus extends from almost beneath the center of the brain, where it attaches to the pharynx proper, to a point near the middle of the prothorax, where it merges into the esophageal valve. In the esophagus the intima is formed as a direct continuation of the dorsal and ventral plates of the pharynx, but being very much thinner it is only barely visible under high-power magnification. The epithelial layer is extremely thin, but its cell walls may still be distinguished in good preparations. A few strands of longitudinal muscle extend the length of the esophagus, but these are very small. The circular muscles, basement membrane, and peritoneal membrane are either absent or too delicate to be visible. Four small muscles support the esophagus just back of the brain. Two tracheal trunks run alongside the esophagus through the circumesophageal connectives.

The esophageal valve is very simple and is well shown in Figure 2, B. At the junction of the mid-gut and fore-gut a ring of one or two cells secretes a peritrophic membrane which is short and disappears entirely only a very short distance below the entrance of the mid-gut. Except for these few cells there is an abrupt change from the structure of the fore-gut to that of the mid-gut.

The mid-gut extends from the esophageal valve into the posterior third of the abdomen with few curves and showing no definite morphological divisions exteriorly. The anterior part is usually much more distended than the posterior part and occupies the bulk of the body space in that region. There is a distinct coil to the right just above the pyloric valve in most specimens. The contents of the mid-gut have been studied and will be described later. Histologically, the mid-gut consists principally of large columnar epithelial cells which vary somewhat in size and shape, according to the distension of the stomach and the age of the cells. A loose lattice work of small strands of circular and longitudinal muscles covers the entire mid-gut, the former being on the inside. The longitudinal muscle strands are relatively few. There seems to be no peritoneal membrane. The enteric epithelium just below the esophageal valve consists of fairly regular cells with large nuclei, each cell with a distal striated hem. Farther down in the stomach wall the cells when they are young and small still possess this hem; as they get older they appear to lose it before discharging their secretory contents. These older cells give off droplike protrusions which extend into the lumen of the gut and later break off and discharge their contents. Relatively few small, young cells have been noticed, so that it is probable that secretion by any one cell is more or less continuous. In the living material the cells of the epithelium are often filled with what appear to be oil globules, which very often are seen to take up green coloring matter, presumably chlorophyll. This same color often appears in the cells of the fat bodies. In preserved material and sections these oil globules show as vacuoles of varying size. There seems to be no correlation between the size of these globules and the age of the cell or the condition of the individual. In many of the cells of some specimens there occur bodies of varying size which stain a deep black in iron haematoxylin. The possible nature of these will be discussed later. The remaining cytoplasm is fairly uniform. There seem to be no distinct differences or divisions in the kinds of

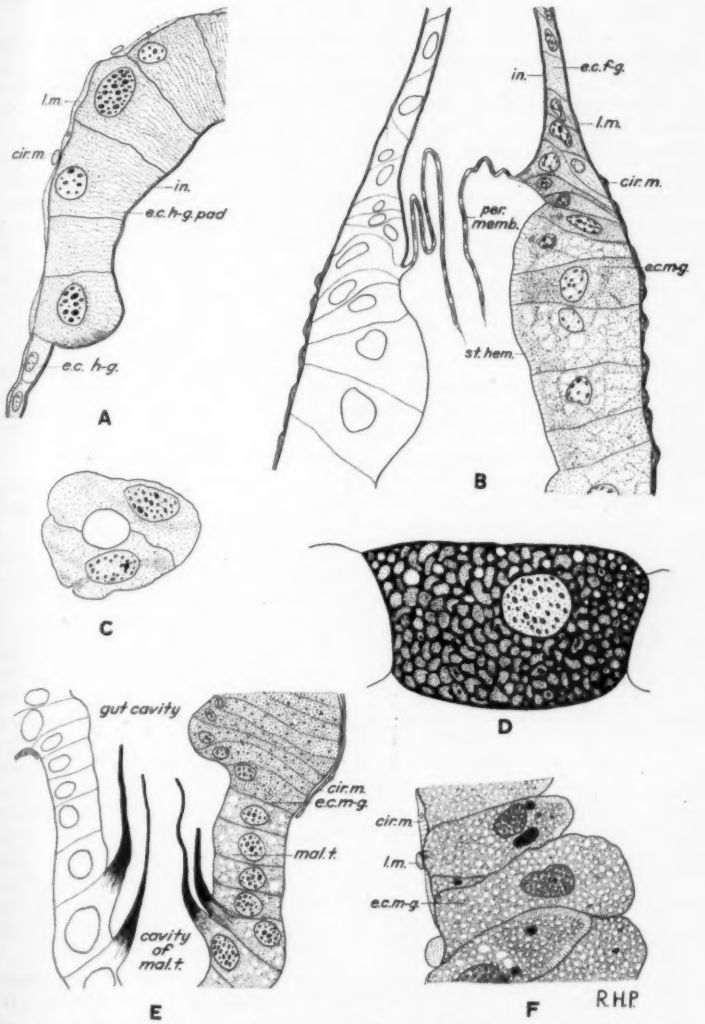

FIGURE 2.—Anatomical details of the cotton flea hopper, all about × 893. A, Longitudinal section of cells of hind-gut pad and hind-gut wall; *cir. m.*, circular muscle; *e. c. h-g.*, epithelial cell of hind-gut; *e. c. h-g. pad*, epithelial cell of hind-gut pad; *in.*, intima; *l. m.*, longitudinal muscle. B, Longitudinal section of esophageal valve; *cir. m.*, circular muscle; *e. c. f-g.*, epithelial cell of fore-gut; *e. c. m-g.*, epithelial cell of mid-gut; *in.*, intima; *l. m.*, longitudinal muscle; *per. memb.*, peritrophic membrane; *st. hem.*, striated hem. C, Cross section of one of the anterior accessory salivary glands. D, Longitudinal section of a cell of the anterior salivary gland. E, Cross section of a part of the pyloric valve, showing entrance of a Malpighiantubule; *cir. m.*, circular muscle; *e. c. m-g.*, epithelial cell of mid-gut; *mal. t.*, Malpighian tubule. F, Cross section of several cells of the mid-gut wall; *cir. m.*, circular muscle; *e. c. m-g.*, epithelial cell of mid-gut; *l. m.*, longitudinal muscle

cells found in any part of the mid-gut; they are uniform in appearance throughout unless changed by distension on account of the gut contents. Near the entrance of the Malpighian tubules the strands of muscle seem to be more numerous.

The pyloric valve is quite simple in this species. Just anterior to where the Malpighian tubules enter the alimentary tract a ring, two cells thick, of the enteric epithelium projects inward and upward. Between this place and their entrance the mid-gut is somewhat constricted. There seem to be no other structures of note in the valve. The structure of the entrance, however, shows some things of particular interest. The four Malpighian tubules join the gut at about equal distances around its circumference. Five or six cells back from the entrance of each tubule there are two circles of cells which give rise to chitinous projections, having much the appearance of the "flame cells." (Fig. 2, E.) Unlike the intima of the hind-gut, these projections stain a deep black with iron haematoxylin, as in the heavily chitinized stylets and pharyngeal plates. The projections have the appearance of being definite secretions of each of the cells and are long enough to extend out through the opening of the tubules and back into the lumen of the hind-gut.

The wall of the hind-gut, except for the hind-gut pad, shows very little difference in structure throughout its entire length and almost no external morphological differences. This portion of the alimentary canal extends from the opening of the Malpighian tubules to the anus, which opens on the ventral part of the last segment. The posterior part of the hind-gut is often somewhat constricted to form a kind of rectum, but there is no difference in the character of the wall. The hind-gut pad consists of a circular disk of tissue surrounding the pyloric valve just below the entrance of the Malpighian tubules and lies mostly on the dorsal side of the hind-gut. (Fig. 1, A.) The cells which compose it are relatively very large and contain large basal nuclei. (Fig. 2, A.) The cytoplasm is fibrous on the distal two-thirds of these cells and has a striated appearance under the microscope. The chitinous intima is somewhat thicker here than in the remainder of the hind-gut. The function of these cells is problematical. The remainder of the wall of the hind-gut consists of small cells similar to but more delicate than those of the fore-gut, with chitinous intima on the inside and a latticework of circular and longitudinal muscles on the outside. These muscles extend over the hind-gut pad. The circular muscles here are on the outside.

SALIVARY APPARATUS

The salivary glands are two roughly spindle-shaped structures lying on each side of, and dorsal to, the beginning of the mid-gut. A constriction, and internally a partition, divides each into an anterior and a posterior part. The size of the gland varies greatly in different specimens, apparently due to the increase or decrease of secretion and its subsequent use. When the glands are distended with the salivary secretions they extend even into the head above the brain and far back into the abdomen. The more usual conditions are represented in Figure 1, A and B. Fastened to the anterior end of each gland is a slender muscle which attaches it to the exoskeleton on each side above the brain. The cells of the salivary gland are very large, roughly hexagonal to square, with large nuclei.

(Fig. 2, D.) The cytoplasm takes a very dense stain with haematoxylin or other nuclear stain. The nuclei are lighter but contain darkly stained granules. In the dense cytoplasm are areas which are much lighter or even clear. When the gland is greatly distended the cells lengthen and widen, but are thinner, so that the nuclei protrude out into the lumen, though they are still covered with a thin layer of cytoplasm. This gives these cells a striking appearance; they differ greatly in general outline from the same cells when the gland is not distended. The secretion in the cavities shows up prominently in sections and is different in the two divisions. Throughout, the secretion is characterized by small droplets embedded in a material which takes up a stain. In the anterior part the secretion takes a nuclear stain; in the posterior part the secretion stains as does the cytoplasm. In addition the anterior part contains some larger vacuolated droplets which sometimes hang together in chains and have the general appearance of growing yeast cells. Although appearing to be without structure, they stain darker than the remaining secretion. (Fig. 3, A and B.) These vacuolated droplets are never seen in the posterior part of the salivary gland.

The salivary duct opens into both divisions of the salivary gland from the ventral side in the region of the partition. The exact course of the duct varies considerably in different specimens, but usually it is as shown in Figure 1, B. From its opening the duct passes forward dorsally to the alimentary tract to about the anterior end of the salivary gland, then posteriorly along the side of the mid-gut to a point about even with the middle of the posterior gland. Here it makes several coils upon itself, is joined by the accessory salivary glands, and passes forward toward the head again, around the circumesophageal commissure and beneath the optic nerve. Under the pharynx it is joined with the duct on the other side, which, in a very short distance, empties into the salivary pump. (Fig. 1, C.) In sections the salivary duct has the appearance of an intracellular tube and is lined with a chitinous membrane or has here at least a very thick cell wall. In longitudinal sections the nuclei are seen to alternate so that the duct forms a sinuous tube among them. In cross section the nuclei appear decidedly eccentric. (Fig. 1, H.) They are much fewer in number as the duct approaches the salivary pump. The cell walls are indefinite.

Three of what appear to be accessory salivary glands or reservoirs join the duct in the position shown in the figure (fig. 1, B), as previously described. Two of these tubular accessory glands pass forward and are often wound about the salivary duct; the third extends posteriorly for a distance varying in different insects and is frequently enlarged greatly to form a thin-walled disklike sack which is closely applied to the mid-gut. The other branches also vary somewhat in size and shape. In cross section these accessory glands usually have several cells and more than one nucleus visible in the same section. The cytoplasm of these cells (fig. 2, C) usually contains vacuoles and is much less dense than that of the cells of the salivary duct proper, which sometimes seem to have a pigmented area around the periphery. The center of these accessory glands rarely contains any secretion which stains definitely.

The salivary pump, which connects with the united salivary duct, lies immediately beneath the anterior end of the pharyngeal pump.

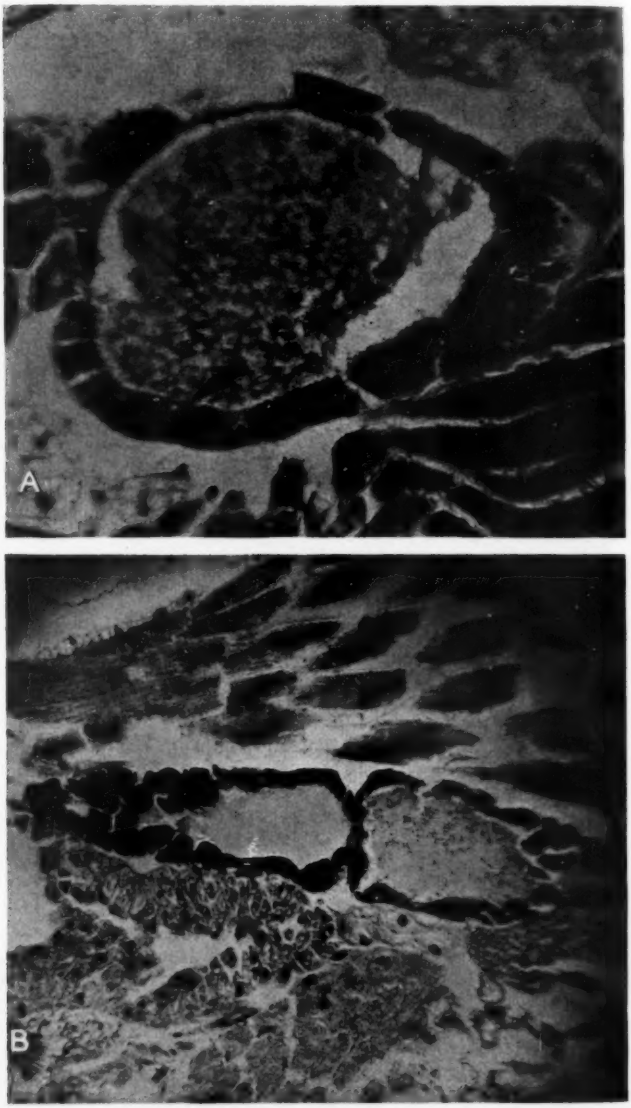

FIGURE 3.—A, a longitudinal section of part of the anterior part of the salivary gland of Psallus, showing the character of the secretion. Iron haematoxylin and orange G; B, a longitudinal section of the salivary gland of Psallus, showing the nature of the secretion of both parts. Ehrlich's haematoxylin and orange G.

It consists of a bell-shaped chitinous organ fitted against the proximal end of the hypopharynx. In the base or proximal end of the bell-shaped part is inserted the plunger of the pump, as shown in Figure 1, C. A chitinous tendon extends through the mass of muscles which work the plunger. These muscles at their middle are divided into two sections, each of which fastens to the head capsule just beneath the eyes. The salivary duct passes forward ventrally and empties into the front of the pump chamber. The dorsal lip of the opening of this duct probably acts as a valve when the plunger goes down, thus forcing the secretion out through a channel in the hypopharynx. A projection from the dorsal wall of the pump extends forward toward this efferent salivary duct and may act in some manner as a valve. The tip of the hypopharynx carrying the efferent salivary duct extends down into the ejection canal formed by the maxillae.

MECHANISM OF MOUTH PARTS USED IN PIERCING AND SUCKING

The mechanism of piercing and sucking has been thoroughly discussed by Grove (*4*) in a paper on *Psylla mali*, in which the various theories of previous writers as to how these things are accomplished have been thoroughly reviewed. Many of his statements on this homopteran may be applied here, except that the actions in this case are less involved. The insect by preference feeds on the more tender parts of the plants and, in the case of cotton, often chooses the red pigment glands. When the insect is ready to feed it elevates the front of the body and brings the beak perpendicular to the plant surface. The area is explored with the tip of the labium, and then the head is forced toward the surface of the plant. The labium bends caudad with the second joint from the base, forming at the point of the "V" an angle of about 30°. The stylets form the front of this V and extend through the other two sections of the labium, which, according to the writer's observations, never release them. The stylets are removed from the plant merely by raising the head, and another place for feeding is quickly chosen. As a rule the insects do not feed long in one place, but occasionally one is seen to remain in position several minutes. Often what are evidently punctures for exploration are made, and the stylets are driven in only a short distance and withdrawn. The entire beak is quite flexible, and a surprisingly large area can be investigated by means of the tip of the labium without further movement by the insect. In a study of sections of the plants on which the insects have fed, it has been noticed that the beak rarely penetrates farther than the first cells of the xylem.

The action of the salivary pump is relatively simple and in this insect is similar to that described for other Hemiptera. The large salivary pump muscles serve to pull back the plunger, thus creating a partial vacuum in the chamber and drawing in the salivary secretion. The elasticity of the chitinous membrane at the juncture of the plunger and the pump wall proper is probably sufficient to force the plunger back into place, close the inlet of the salivary duct, and force the salivary secretion out into the ejection canal through the hypopharynx. The secretion then passes out through the ejection

canal of the stylets. In life these insects may often be seen with a drop of semiopaque liquid, which is probably the salivary fluid, at the end of the beak.

The factors involved in drawing out the sap from the host plant along the suction canal, as they have been stated by Grove and others, may be briefly reviewed thus: (1) Capillary attraction up through the suction canal; (2) pressure of the sap or turgidity of the cells; (3) the pumping action of the pharynx accomplished by the raising of the dorsal pharyngeal plate, probably in a series of waves starting at the anterior end; (4) the peristalsis in the esophagus; and (5) the action of the esophageal valve. Although there seems to be no definite organic connection between the end of the pharynx and the suction canal, it seems probable from the size of the muscles and from the constancy of their appearance throughout the order that the pumping action of the pharynx is the principal factor involved. It is possible that the direction of the pumping force may be reversed, driving a secretion out of the gut into the plant in addition to the salivary fluid; but from the presence of a definite esophageal valve, although this is somewhat reduced in complexity, and from the lack of visible secreting cells of any size in the fore-gut, this would not appear to be a normal occurrence in this insect.

PARASITES

During the course of the study of the anatomy of the insect, parasites have been looked for which might possibly cause the deformation of the plant tissue. By making a smear of the entire mid-gut as soon as it is removed from the insect, it is possible to find a few coccus forms of bacteria. These are relatively scarce and are no more than might be expected, having been described in other insects in the same situation, and their presence here is probably not of importance in the general problem under discussion. Jenner's blood stain was used on these slides. The presence of some dark staining bodies has been described in the enteric epithelium of the mid-gut. (Fig. 2, F.) Three things with regard to these seem to point to their being metabolic products rather than parasites: (1) They vary greatly as to size and shape; (2) it is impossible to demonstrate anything but a homogeneous composition by means of stains; and (3) these bodies are found in the lumen of the mid-gut and hind-gut from the point where they begin to occur in the epithelium, about opposite the first abdominal segment, on through to the end of the gut in apparently undiminished size and number. It is suggested that these may be excretion products from these cells. The character of the secretions of the salivary gland in this insect has been described previously and presents some points of interest. The appearance of the smaller emulsoid bodies throughout the cavity of both the anterior and posterior division of this gland probably may be taken as the natural appearance of the staining reaction of the secretion in this insect. The appearance of the large globules in the anterior salivary gland is striking and peculiar in that it is limited to this section of the gland and was present in practically all the specimens examined, some 60 in all. (Fig. 3, A and B.)

STUDIES OF PRESERVED PLANT MATERIAL WITH RESPECT TO THE EFFECTS OF THE BITE OF THE COTTON FLEA HOPPER

NATURE AND EXTENT OF COTTON FLEA HOPPER INJURY ON COTTON

So far as the external appearance goes, the effect of the Psallus bite upon cotton is threefold: (1) It causes a shedding of the young flower buds, or squares; (2) the number of internodes is increased while their length is decreased; and (3) conspicuous swellings appear at intervals over the whole plant but seem to occur either near, or exactly at, the point of the bite, the number of these bites and the persistence of the swellings giving rise to the apparent systemic condition. As a result of the first two, the entire habit of the plant is much changed, so that "hopper plants" may easily be recognized in any field. These plants are further characterized by having short branches excepting the lower two or three, which are often long; the plant itself is tall and spindling. The swellings appear on all parts of the stem, petiole, midrib, and the main veins of the leaf.

EXPERIMENTS ON HOPPERS AND ON LIVING COTTON PLANTS

During the summer of 1925 a number of experiments were undertaken at Port Lavaca, Tex., to determine whether the insect carried a plant disease or, if not, what were the main factors producing the result of the insect bite. Since material from these experiments has been used in the present study it is necessary to review the results of this work.

The experiments were principally attempts to duplicate the work of the hopper by artificial means. As the summer's work advanced it became more evident that the effect of the hopper's bite was quite local and apparently not transmitted through the plant itself from one part to another. So, while the first problem was kept in mind, the later work was directed toward the isolation of other possible factors involved in the production of abnormal plants as a result of hopper bite.

INOCULATIONS AND FIELD EXPERIMENTS

Methods

The plants used in all experiments were chosen in the field. A certain amount of protection against hoppers was afforded by dusting the plants with sulphur, and in certain experiments by covering them with sacks. The work was somewhat handicapped in some phases by the lack of plants entirely free from all insect injury. The large number of plants used made it impossible to provide cages.

At first, three methods of inoculation were used: (1) Hypodermic injections in various parts of the plants; (2) cuts with a safety-razor blade dipped in various solutions; and (3) punctures with a dissecting needle likewise dipped in the solutions. Later, owing to the fact that the mechanical disturbance of the plant tissue obscured the effect of the solutions, these methods were largely replaced by the use of punctures made with pins known as "minuten Nadeln." The pins were set in a small piece of softwood, mounted on a handle for convenience in using, the tip of the handle being dipped in shellac to fix the pins in place. After each set of inoculations all instruments used were sterilized in boiling water and 90 per cent alcohol, and the minuten Nadeln apparatus was again dipped in shellac. The places of inoculation and other special methods used are described under

each experiment. In all cases where hoppers were used in the experiments, from 100 to 150 of the insects were crushed in about 3 c. c. of rain water.[6] In most cases notes were taken at 24-hour intervals.

The First Series of Experiments

G. F. White and K. P. Ewing started the first series of experiments with 70 cotton plants before the writer's arrival. Later some of the inoculations and notes were made by the writer. In these experiments the razor blade, dissecting needle, and hypodermic needle were used on leaf veins, leaf petioles, square stems and branches, the main stem at various places, and the root crown. Suspensions of crushed hoppers, croton juice, crushed diseased cotton, and crushed mint were injected. None of the results were clear-cut, but some differences in the way the lesions healed were noticed. There was no evidence of any systemic disturbance of the plant or any more shedding of the squares than occurred among the checks, hoppers being present in small numbers in the field.

The Second Series of Experiments

In the second series 60 cotton plants were inoculated in a series of 5 experiments of 12 plants each, solutions from croton, mint, and diseased cotton juice, and suspensions of crushed hoppers from croton and from cotton being used. Inoculations for each experiment were as follows:

Plant 1 (check): Water injected with a hypodermic needle.

Plants 2, 3, and 4: Solution injected with a hypodermic needle into the base of the terminal bud and on two upper square stems.

Plant 5 (check): Safety-razor blade dipped in water, and slits made in two upper leaf petioles and two upper square stems.

Plants 6, 7, and 8: Razor blade dipped in solution, and inoculations made in places similar to those of the check.

Plant 9 (check): Pin punctures (minuten Nadeln), two groups of three on each of two upper leaf petioles and on a second square stem, and one group of three on the upper square stem, the groups of three being a stated distance apart, the pins having been dipped in water.[6]

Plants 10, 11, 12: Pin punctures made as in check; pins dipped in solution.

None of the plants, except those treated with crushed hoppers from croton, showed any constant, definite distinction between the checks and the inoculated plants. In the case of the checks injured with a razor blade the cuts were clean and healed with a tan color. In the plants inoculated with hoppers from croton there was a growth of distinctive whitish cells from the sides and bottom of the upper leaf petiole of one, the lower leaf petiole of another (slight), and from all the cuts on a third plant. In the case of the pin punctures the checks showed dark holes at the points of puncture, sometimes with a slight swelling about them. In the plants inoculated with hoppers certain punctures became enlarged and burst open, showing a tan-colored

[6] Distilled water free from metallic impurities was not available where the experiments were carried on. In view of this fact and in the absence of sterile plants in the field, it was necessary to depend on a difference in reaction between plants inoculated with rain water and those inoculated with other substances in rain water, rather than to expect the checks to be entirely free from injury. Rain water was therefore used throughout the experiments.

granular tissue beneath the skin. These slits extended sometimes for a millimeter or more on each side of the puncture. This condition appeared in many of the punctures on all three plants inoculated, especially on the leaf petiole and second square stem. The slits appeared between 48 and 72 hours after the time of inoculation. A single puncture on one of the plants inoculated with mint had a somewhat similar appearance. These developments are referred to in this paper as split lesions.

Injections of Suspensions of Cotton Buds on Which Hoppers Had Been Caged

A hundred hoppers were caged on the extreme tip of each of three cotton plants for 24, 48, and 72 hours, respectively. At the end of that time the tips were removed, crushed in 1 c. c. of water and injected into 10 plants by pin punctures in the two upper leaf petioles and two upper square stems. A fourth plant was protected from hoppers for three days, examined and found free from hopper damage, and was crushed and injected as a control. The results are given in Table 1.

Table 1.—*Effect on cotton plants of injections of rain water containing soluble matter from crushed tips of cotton plants on which Psallus had been feeding, Port Lavaca, Tex., July, 1925*

[Ten plants were inoculated on each date and 21 inoculations were made per plant]

Date inoculated	Period tip was exposed to feeding of Psallus	Average height of plants July 10	Plants showing split lesions	Total split lesions	Average plant growth during—	
					First week after July 10	Second week after July 10
1925	*Hours*	*Inches*	*Number*	*Number*	*Inches*	*Inches*
July 7	24	18.05	8	40	4.2	4.75
July 8	48	19.95	10	45	3.2	4.85
July 9	72	17.15	10	62	3.4	4.55
Do	(a)	20.65	7	16	4.4	4.40

[a] Check tip; suspension was made from an uninfested tip.

In addition to the split lesions, similar to those caused by inoculation with crushed hoppers, there was a difference in the average rate of growth which was paralleled by results with hopper cages; i. e., a slowing up of the growth when hoppers were first introduced, followed by a slight increase.

Effects on Cotton Plant of Continuous Exposure to Suspensions of Crushed Hoppers and Hoppers Caged on Localized Areas

Eight cotton plants in the field were protected from hoppers for over a week. After the experiments were started, however, some hoppers hatched out in two of the plants and caused hopper squares [7] before they were found and killed. Cups of adhesive plaster were made about the stems of three of the plants; one of these was filled with water to serve as a check, two were filled with crushed hopper suspension, and slits were then cut in the stem below the level of the

[7] The term "hopper squares" is used to designate those squares which when very small darken and die in a way which is typical of the injury produced by *Psallus seriatus* and related insects on cotton.

liquid so that the plants might continually take up the solution from the cups. The liquid remained about the check and one treated plant for 12 hours and about the other plant for 48 hours. Two plants, one of them the check, on which live nymphs were found, showed hopper squares. The other plant showed no typical hopper squares. The pressure of the sacks over the plants may have caused some of the squares to die. Table 2 summarizes the results.

TABLE 2.—*Effect on cotton plants of exposing the stems to suspensions of crushed cotton flea hoppers, applied July, 1925, Port Lavaca, Tex.*

Plant No.	Period of exposure	Height at time of inoculation	Growth during—		Dead squares found during the experiment
			Week preceding inoculation	Week following inoculation	
	Hours	*Inches*	*Inches*	*Inches*	*Number*
104	12	20.5	6.5	3.0	0
105 (check)	12	25.0	6.5	3.5	1
106	48	23.0	7.5	2.5	[a]6

[a] The pressure of the sack over the plant may have caused some of the squares to die.

Five plants were used in a test to determine the effect of hoppers confined on a limited area of the plant. One of these was used as a check, and on the other four were fitted vials from which the bottoms had been removed. Into these vials 15 hoppers were introduced every other day and there confined so that they could feed only on the area beneath the vial. The vials were placed as close to the terminal bud as possible. Data concerning these plants are given in Table 3.

TABLE 3.—*Effect of confining cotton flea hoppers on limited areas of cotton plants, Port Lavaca, Tex., 1925*

Plant No.	Height of plant at beginning of experiment, July 10	Total growth of plant from July 10 to 17	Distance from center of vial to tip of plant on July 17	Growth of plant from July 17 to 24, measured from center of vial to tip	Total growth of plant from July 24 to August 1 [a]
	Inches	*Inches*	*Inches*	*Inches*	*Inches*
107 (check)	20.5	4.0	----------	[b]5.00	1.50
108	19.5	3.5	1.3	1.65	1.05
109	13.0	6.5	1.0	1.00	0
110	11.5	3.5	1.9	1.80	1.50
111	19.0	5.0	2.6	2.10	.50

[a] Limited growth is probably due to pressure of inclosing sacks.
[b] Total growth of plant in this one case.

The plants showed absolutely no hopper squares and little, if any, difference in growth. The only evidences of the presence of the hoppers were the lesions beneath the vials.

CHEMICAL INTRODUCTIONS AND INOCULATIONS FOR THE STUDY OF LESIONS ON COTTON STEMS

Commercial diastase, pepsin, trypsin, and invert sugar sirup solutions were each introduced into five plants. On each plant the introductions with minuten Nadeln pins were in two groups of three on each of the two upper leaf petioles and second square stem and one

group of three on the upper square stem. The checks were five plants similarly treated with water. At the same time, partly to serve as checks and partly to serve in a study of lesions, five plants were inoculated from 17 to 21 times with crushed-hopper suspension, in groups of three each at intervals of about one-half inch. Table 4 gives a summary of the two experiments.

TABLE 4.—*Effect produced on cotton plants by injections of various chemicals and of crushed cotton-flea-hopper suspensions, Port Lavaca, Tex., 1925*

Material injected	Injections per plant	Plants showing split lesions	Total lesions	Average height of plants at beginning of experiment	Average growth per plant during first week after treatment
	Number	*Number*	*Number*	*Inches*	*Inches*
Not injected (check plants)	21	2	2	12.5	4.7
Diastase	21	5	30	10.1	3.6
Pepsin	21	3	11	10.6	3.2
Trypsin	21	1	2	11.3	3.9
Invert sugar	21	0	0	13.5	3.1
Not injected (check plants)	54–63	3	* 28	14.7	3.2
Crushed-hopper suspension	54–63	5	111	12.2	2.9

* Nearly all on one plant.

Diastase was the only reagent which showed any results comparable with those from the inoculation of crushed hoppers. With regard to the other plants, nearly all of the split lesions were in the third, fourth, and upper part of the fifth internodes below the bud; in other words, at about the place where the cells were ceasing to grow. This method of inoculation on the main stem seemed best.

Inoculations of Centrifuged Croton Juice

A large number of tips of *Croton engelmanni* were crushed in water and centrifuged for five minutes. Only chloroplasts and particles of other plant tissue were thrown down. The yellowish liquid above was drained off, and five cotton plants were inoculated with it on the main stem at intervals of about 1 cm. Five plants were similarly inoculated with the thicker part of the solution. None of these plants showed a single lesion comparable with those caused by the crushed-hopper suspension.

Inoculation of Croton Plants

A crushed-hopper suspension was used in inoculations on the main stem of four croton plants. The plants showed a slight darkening in the region of the inoculation, but neither swelling nor split lesions.

Inoculations of Cotton Plants with Suspensions of Crushed Hoppers

A large number of hoppers were crushed in water and that filtered through a Berkefeld filter No. W–20, 10½; the filtrate was then introduced into the main stems of five cotton plants. The residue was introduced, unfiltered, into the main stems of five other cotton plants. In order to determine the effect of letting the suspension stand for some time before inoculating with it, five plants were

inoculated in the main stem with freshly crushed-hopper suspension and three hours later five more plants were inoclutaed with the same suspension. The results of these two experiments are presented in Table 5.

TABLE 5.—*Number of split lesions produced by inoculating cotton plants in various ways with suspensions of crushed flea hoppers, Port Lavaca, Tex., 1925*

Material injected	Plants	Inoculations per plant	Split lesions
	Number	*Number*	*Number*
Filtrate from crushed hoppers *	5	54–63	7
Residue left after filtering the suspension of crushed hoppers *	5	54–63	45
Unfiltered suspension of crushed hoppers *	5	54–63	36
Suspension of crushed hoppers, 3 hours after preparation	5	54–63	3

* Freshly prepared.

It is noticeable that the number of split lesions was less on these plants which were large than on the younger plants used earlier in the season from this same patch with the same number of inoculations. (Table 4.)

INTERMITTENT INOCULATIONS

This experiment was started as soon as the late-planted young cotton began to put on squares. From August 7 to September 11, 10 plants were given twice a week from 3 to 9 inoculations with crushed hoppers on the two upper internodes of the main stem and on each branch. Five plants were similarly treated with water. These 15 plants and 5 others for comparison were measured twice weekly. During the first week in September the inoculations were discontinued on 1 check plant and on 4 of the other plants to test the recovery of the plants from the effect of the inoculations. Toward the end of the experiment from about 150 to 200 inoculations per plant were given twice a week. Table 6 shows the differences in plant heights. Four plants were eliminated during the experiment, owing to injury by the bollworm (*Heliothis obsoleta* Fab.).

TABLE 6.—*Effect on growth of cotton plants of repeated inoculations with crushed flea-hopper suspensions during fruiting period, Port Lavaca, Tex., 1925*

Treatment of plants	Plants treated	Average height of plants		Growth of plants from Aug. 28 to Sept. 11
		Aug. 7	Sept. 11	
	Number	*Inches*	*Inches*	*Inches*
Check (untreated)	5	6.9	31.0	9.4
Inoculated Aug. 7 to Sept. 11 with suspension of crushed hoppers	3	7.5	23.6	7.0
Inoculated Aug. 7 to Sept. 11 with water	3	7.5	24.5	6.6
Inoculated with suspension of crushed hoppers Aug. 7 to Sept. 1; recovery Sept. 1 to Sept. 11	4	7.6	25.3	8.0
Inoculated with water Aug. 7 to Sept. 4; recovery Sept. 4 to Sept. 11	1	7.5	25.5	8.0

In addition to differences in height, all the inoculated plants showed an average shortening of the internodes by about one-third of their length. All plants made a low scrubby growth with numerous branches, often two at each internode. In all respects save in the

appearance of lesions there was no difference between the crushed-hopper and the check inoculations. Hopper squares appeared on all plants in about equal numbers. There is little doubt that in all cases this injury of the squares was due to the presence on the plant of hoppers and other mirids.

The results of the experiments reported in Tables 1 to 6 may be summarized as follows: (1) Lesions could be studied best when inoculations were made up and down the main stem at short intervals; (2) there was no evidence in any experiment of persistent systemic disturbance transmitted very far from the point of inoculation; (3) positive results were two, the occurrence of swellings at the points of inoculations with a split showing peculiar abnormal tissue later on, and a shortening of certain of the internodes which were inoculated when young; (4) the latter occurred about as often in control plants as in inoculated ones and was least common on vegetative branches, like those coming out near the bottom of the plant; (5) swellings followed by split lesions occurred rarely on the control plants and were of some significance as they appeared similar to lesions caused by the hopper; (6) these split lesions occurred in numbers on cotton plants receiving inoculations with (*a*) crushed-hopper suspension, (*b*) crushed cotton tips on which the hoppers had been caged in large numbers, and (*c*) diastase solution; (7) a split lesion consisted of a decided swelling appearing around the puncture which about 48 hours thereafter split, sometimes as much as 4 mm., exposing a tan granular material beneath, which became darker with age; (8) so far as was visible externally, plants showed a complete recovery from any and all inoculations, except in the region of lesions, which did not develop further; (9) there was a decided variation in the response of different plants, both as regards number and size of lesions and shortening of the internodes at the sites of lesions; (10) lesions appeared to develop best on internodes 3 to 5, inclusive, on the main stem, although they appeared at almost any point inoculated, that is to say, they appeared usually at the place where the cells were just ceasing their growth; (11) croton plants did not show a response similar to cotton when inoculated with crushed hoppers; (12) hoppers might be caged on cotton plants and permitted to feed close to the buds without causing a shedding of squares; and (13) suspensions of crushed hoppers which had been filtered or allowed to stand for several hours gave few or no lesions when injected into the cotton plant.

LABORATORY EXPERIMENTS

Vital Dye Experiments

An effort was made through the use of vital dyes to determine whether the hopper transferred material from one plant to another. The method used was to place the petioles of young leaves in a solution of the dye until the veins were colored, place hoppers on these leaves, and after a short time transfer the hoppers to plants which had been deprived of chlorophyll by being kept in the dark. Aqueous solutions of the following stains were used: Eosin, Bismarck brown, Congo red, aniline green, gentian violet, acid fuschin, neutral carmine, methylene blue, neutral red, and several mixtures of two of the above. All the dyes went up into the plant tissues to some extent, gentian violet least, and methylene blue, eosin, and neutral red most. During

the course of the experiments the hoppers were examined by dissection, and methylene blue, eosin, and aniline green were observed in the gut and Malpighian tubules. The green stain also caused the gut wall to appear opaque. The eosin caused the death of the hoppers in from two to three hours. Upon examination after death the dye was found in the Malpighian tubules; before death it was seen in the gut. No stain was ever seen in the second plant on which the hoppers were subsequently caged, but these experiments led to those described below.

Nature of the Effect of Hopper Bite on the Pigment Spots

In the work with hoppers and vital dyes it was noticed that places on leaves where the hoppers had fed retained, when the leaf was placed in Carnoy's solution,[8] the red pigment spots or oil glands (*9*) normally characteristic of the cotton leaf, whereas the untouched spots were dissolved out. In the experiments with this reaction the bottoms were removed from vials and replaced with stiff paper in which openings of a definite size and shape had been cut. Hoppers in varying numbers were placed in the vials for varying lengths of time, while the opening which had been made in the paper bottom of each vial was placed successively over different parts of the plant.

The effect of the bite of hoppers on pigment spots did not spread outside the area exposed to them, no matter where the hoppers were placed. The material on which the hoppers had fed was fixed in Carnoy's and in Bouin's solution for microscopic study of the areas affected. In an attempt to determine the nature of the reaction, a number of other sucking insects feeding on cotton were caged on cotton leaves in a similar manner. The aphids and two species of Cicadellidae that normally fed on cotton gave no results similar to those from the hoppers, though relatively few of these insects were used. Three specimens of nymphs of a pentatomid and four different species of Miridae gave, however, a result that seemed identical with that produced by the hopper under similar conditions.

An attempt was made to duplicate the effect of the hopper bite artificially. Stems and leaves were punctured and treated with crushed-hopper suspension and solutions of diastase, trypsin, and pepsin, and allowed to remain overnight. When placed in Carnoy's solution none of the specimens showed a distinct preservation of the spots. Later pieces of leaves and petioles were punctured and placed bodily in suspensions of crushed hoppers and in diastase solution for varying lengths of time. The hopper suspension showed no effect, perhaps on account of its great dilution; but the material soaked four hours in the diastase solution showed the preservation of the pigment spots about the areas of punctures. The checks that had been soaked in water showed no effect. Lack of time prevented the trial of other chemicals.

Summary of Laboratory Experiments

So far as the transference of material, by regurgitation, from plant to plant was concerned, the experiments with vital dyes were negative, but further tests along this line seem warranted.

[8] Owing to lack of absolute alcohol the Carnoy's fixing solution was made up with 95 per cent alcohol 1 part, glacial acetic acid 1 part, chloroform 1 part, corrosive sublimate to saturation. The material was washed in 70 per cent alcohol.

Methylene blue, eosin, and neutral red proved to be the most promising dyes for tests of this kind.

When hoppers were caged on a leaf it was found that the pigment spots or glands about their bites failed to dissolve out in Carnoy's solution, as did the other spots.

Similar results were given by pentatomid nymphs and four other species of mirids under similar conditions.

Leaves punctured and soaked in crushed-hopper suspension gave no results, probably owing to the great dilution.

Leaves punctured and soaked in diatase solution for a short time showed a preservation of spots about the punctures when preserved in Carnoy's solution.

IMMEDIATE EFFECT OF THE HOPPER BITE

The material which was used in the study of the immediate effect of the hopper bite consisted of sections of those leaves on which hoppers had been caged for varying lengths of time and had been restricted in their feeding to a small square area by means of an opening in a paper at the bottom of a vial, as previously described. Whether the insects had fed on the plant 3 hours or 24, there were no apparent differences in the stained preparations. In the cells the cytoplasm showed a tendency to plasmolyze throughout the area on which the insects had fed. The chloroplasts and nucleus seemed to have been broken up and scattered through the cell, giving it a decided homogeneity. Iron haematoxylin stained all of the cell contents very heavily, so that it was impossible to make out details. Other stains, such as Ehrlich's triacid mixture, Ehrlich's acid haematoxylin, and safranin, gave somewhat better results but failed to resolve the cell contents into constituent parts. In this respect all the cells of the leaf in the square area on which the insects had fed reacted similarly. Cell walls in places both inside the leaf and on the outer epidermis had been ruptured in various places. These breaks had the appearance of being made mechanically rather than by any dissolving action. There seemed to be no tendency in this tissue for the effect of the bite to spread beyond the confines of the square in which the insects had fed, the cells on its edge being entirely normal.

In connection with this part of the study, sections were made of leaves which had been punctured and soaked in diastase solution for four hours. These sections in places around the punctures showed a clumping of the cytoplasm and dissolution of the chloroplasts similar to that of the material described above. The cytoplasm of these cells stained a deep black in iron haematoxylin. In one place noted about 15 palisade cells were affected on each side of the point of puncture. This may perhaps be taken as the average of spread in this length of time.

Relatively little could be ascertained concerning the effect of the insect's feeding on the pigment spots or glands. In the area not touched by the insects these appeared as circular openings surrounded by cells of an epithelial nature. In the glands on which the insects had fed or which had been touched by the diastase solution, there was a black staining material in the center of the gland and throughout the surrounding cells. The chemistry and anatomy of these glands have recently been studied by Stanford (*10*). It is interesting to note that the tissue on which the insects had fed was somewhat similar

in reaction and appearance to that found surrounding the beak of certain aphids when this organ was inserted into a plant. This has been described and figured by Wells (*12*) and Biedermann (*1, pp. 808–810*), and it has been shown that this sheath about the proboscis consists of calcium pectate and tannic substances.

A STUDY OF THE SWELLINGS AND RELATED INJURY CAUSED BY THE BITE

Externally the swellings caused by the bite itself showed little variation except as regards size, extent, and amount of splitting. On the plant they occurred on the stems, petiole, midrib, and smaller leaf veins. The swellings themselves were principally the result of an increase in size of the individual cells, and to a very slight extent of an increase in the number of cells. The swelling might take place in all directions but was always more obvious in cross than in longitudinal sections, hence laterally rather than with the axis of the stem. In the center of the swelling, apparently at the place where the insect bites, there was a distinct destruction of some of the cell elements, while others enlarged to take their place. Along the margin of an area of destruction certain cells seemed to have collapsed. Sometimes as a result of this destruction of cells, and at other times apparently without visible cause, there was a displacement of the position of normal cells. This was especially true of the elements of the vascular bundle, the position of the xylem often being changed as shown in Figure 4, A, and the constituent parts broken up as shown in Figure 4, B. The chief change in cell structure took place in the cortex and in the cells recently formed in the cambium, but the phloem was also involved, and less commonly the xylem. The individual cells showed a great diversity in form, usually being somewhat irregular and thus different in shape from those of normal condition.

In certain cases the structures of the cells themselves were changed. The epithelial cells were often found to have grown larger and varied in shape and size, appearing like those of the cortex or even more irregular. Very often certain elements had enlarged and taken the place of destroyed tissue, as is shown in the cross section of the midrib. (Fig. 5, A.)

The most marked change, however, was in the content of the cell wall about an area where the cells had been destroyed, or in a lesion which had split so that the cells were exposed to the air. Here the cellulose had disappeared from the cell walls and pectic material alone apparently survived. This was evident when such a section was examined under polarized light, and it was also shown by stains. This change in structure seemingly gave rise to the characteristic appearance of the split lesions seen in field work. In these structures the swelling split open, and the enlarged cells appeared as a tan granular substance in the wound. In section these split lesions were seen to be lined with dead cells, and the open part often extended back under the epidermis for a short distance in all directions, sometimes leaving pockets of destroyed cells overlaid by living ones. Some swellings which showed no external split had below the epidermis an extensive pocket of dead cells and often relatively large air spaces.

Frequently the entire contents of injured cells were lacking. But when still present there was always a most striking change of appearance in these parts. When stained with iron haematoxylin and

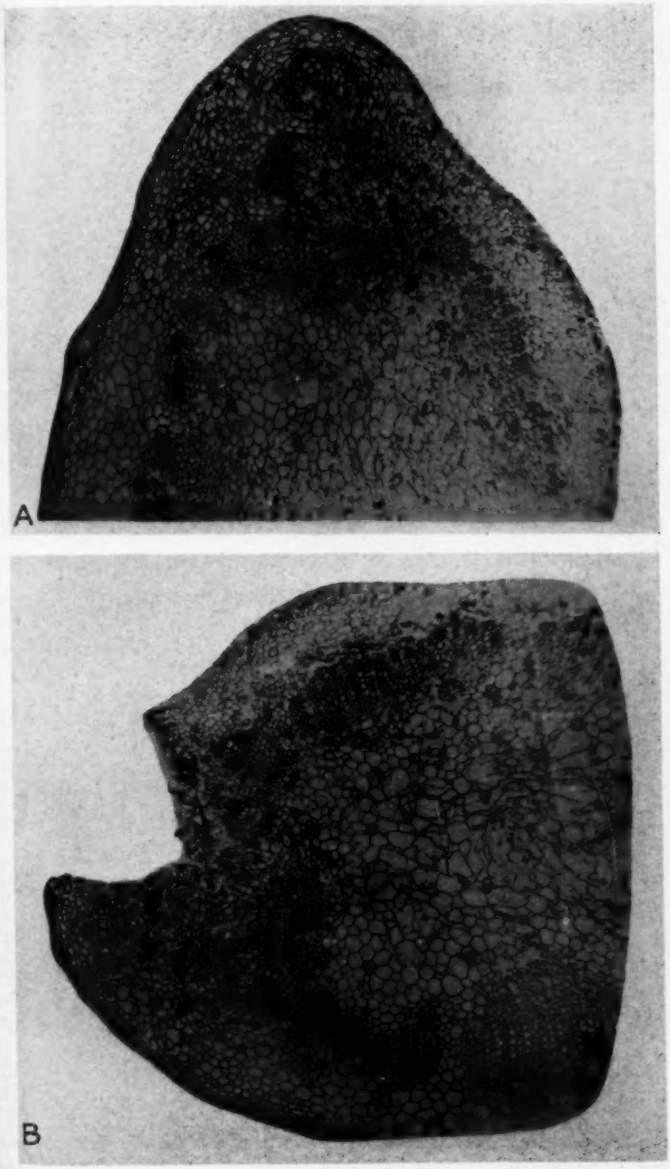

FIGURE 4.—Injuries to cotton by the cotton flea hopper, *Psallus seriatus:* A, Swelling andmisplaced vascular bundle on stem caused by the insect; B, cross section of split lesion on stem caused by hopper bite. Material from hopper cages, Port Lavaca, Tex. All the photomicrographs of plant material used in the illustrations of this article were of sections stained with iron haematoxylin and orange G

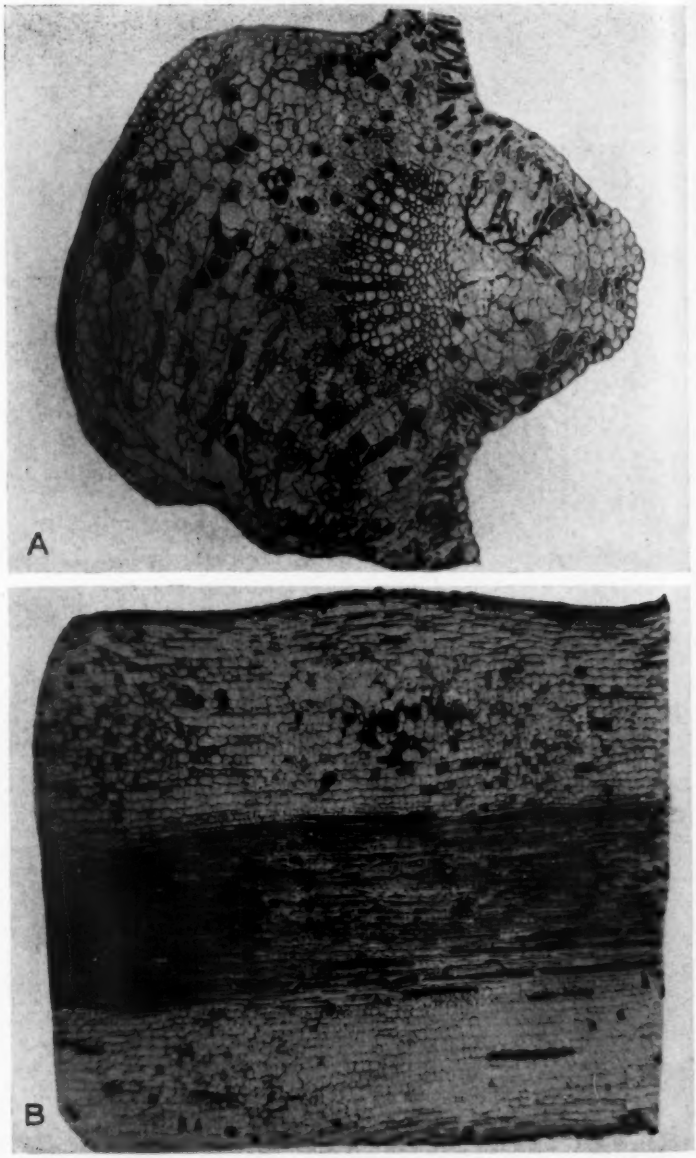

FIGURE 5.—Injuries to cotton by the cotton flea hopper, *Psallus seriatus:* A, Cross section of swelling on midrib of cotton leaf; B, longitudinal section of stem preserved four days after the hoppers had been caged on the plant, showing a series of cells containing granular material which came from near the beginning of the region of the swelling. Material from the hopper cages at Port Lavaca, Tex.

orange G, the cells could be separated into four types, which seemed to merge into each other. Certain cells were filled with a homogeneous material which stained a deep black and showed no trace of nucleus or chloroplasts. Other cells were filled with a homogeneous yellow-staining material but with the nucleus still in the cell and the chloroplasts intact around the periphery. Other cells were filled with yellow particles of the same size in any one cell, but of different sizes in different cells. In certain of this group of cells a nucleus, or the chloroplasts, or both, were still visible. The last group of cells to be noted was that in which the cells contained oval or round particles, often with darker rim, and had a glistening appearance much like spores of fungi. These varied in different cells from the size of the largest yellow particles to about one-half larger. At times they were arranged in a loosely branching series throughout the cells. At other times they were arranged around the periphery of the cells. An average size for the largest was about 0.005 mm. (Fig. 5, A.)

The exact extent of the effect of the bite up and down the stem has not been ascertained. Experiments during the summer of 1925, however, point to the probability that it is fairly local, at least not extending more than two or three internodes from the point of bite. A very interesting fact concerning the material found in the affected cells about the lesions was that the cells involved tend to occur in vertical series, each series often separated from the next by several normal cells. There was a tendency at times to spread subcortically and also around the epidermis.

Material which had been inoculated with crushed-hopper suspension and later preserved and examined microscopically showed throughout effects which were similar in all cases to those due to the punctures by the hopper. The type of swelling and the change in cell structure and in cell contents were closely parallel in both cases. Figures 5, B, and 6, A, show this comparison very well. By microscopic examination it was also found that the method of inoculation with "minuten Nadeln" was quite satisfactory, the pin pricks extending only through the cortex, and hence very little farther than the proboscis of the insect entered.

SHEDDING AND INSECT INJURY

A study of the literature on abscission in cotton shows that two theories have been advanced in regard to the probable cause of the shedding of the squares and bolls. Lloyd (*7*) has brought together considerable material to support his contention that abscission is a result of competition between bolls for water. Mason (*8*) has given support to the theory that the shedding is determined by the rate at which food is elaborated by the plant and is used by the developing fruit. The exact details of the method of abscission have not been completely worked out. It has been shown, however, that abscission is preceded by a growth of cells in the abscission zone, followed by a digestion of the middle lamella and adjacent layers of cellulose wall. Pockets of resin or gum inclosing loose cells are frequently present.

It will be seen from the preceding description of insect injury that the probable nature of abscission due to the insect may support either theory, or both, or neither. It is possible that a change in the cell wall or in cell contents will start the abscission reaction. It has

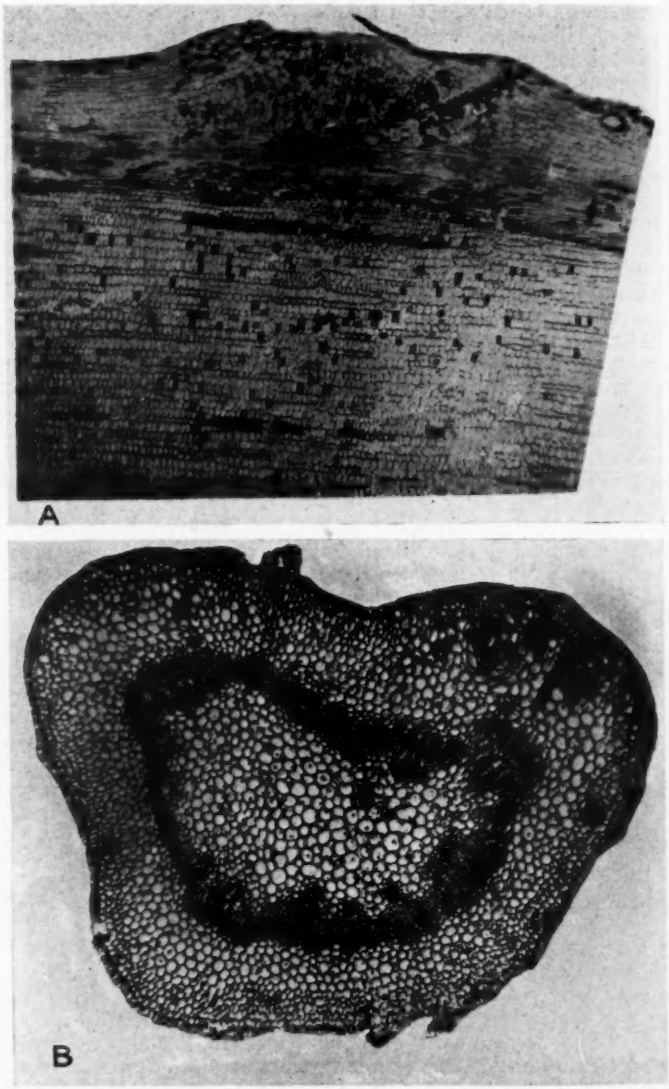

FIGURE 6.—Injury to cotton plant by the cotton flea hopper: A, Longitudinal section of stem through place of inoculation with crushed-hopper suspension, preserved 48 hours after puncture; B, cross section of stem in which the feeding place of the insect appears black, preserved after the insects had fed on the plant for 24 hours

been noted that a destruction of cells sometimes takes place at the site of the insect bite without being visible externally. This actual destruction of cells may in itself be the cause of the shedding of the squares or it may operate to prevent the absorption of water by the developing bud. The change in cell contents may have a similar result.

Preserved material has been examined to see whether it shows any correlation between the length of time during which the insects have been allowed to feed and the changes in the plant tissue. Four days after hoppers have been caged on a plant a cross section of the bud shows all stages of cell secretion, and in a longitudinal section all variations may often be seen in a single row of cells, so that it is impossible to point out any one cell as being the one in which the change originated. On one occasion insects were caged on a bud for 24 hours and then removed and the bud preserved in fixing fluid. On examination sections of this bud showed the cell contents in the condition previously described as containing yellow particles, and in other places the cells contained the black particles. (Fig. 7, A.) Where each locality represented a separate place of bite there was no admixture of the two types of cell contents. Certain cells were entirely emptied of their contents, and in others there was a clumping of the material of the cell, which stained very darkly, paralleling the condition described above. In the stem of this same bud a developing egg was found which was probably that of Psallus. The only evidence of injury in the region about the egg was a collapse of some of the cells. A typical section is shown in Figure 7, B.

CELL INCLUSIONS

In a study of this kind the possibility of the presence and transmission of organisms of a parasitic nature must be taken into consideration. The change in the character of the cell contents is the only fact derived from microscopic study which would point to the possibility of such organisms being present. It seems impossible that these peculiar structures are artifacts due to fixation, since a large number of fixing fluids have been used under various conditions. However, it may be that they are the normal regeneration responses of the plant to a wound of any kind. Other than this, there are four possible explanations for the change in appearance in the cell contents: (1) The structures seen may be gums or resins due to excess food, the products of insect secretion injected into the plant; (2) they may consist of a deposit of material by the plant in direct response to toxins or enzymes injected by the insects; (3) they may represent a developing parasitic organism showing stages of growth, and may be any of the following—yeast, slime mold, fungi, amoebae, or bacteria. In general appearance the diseased portion resembles a similar portion of the club root of cabbage. Certain stages show a likeness to developing yeast, and in this connection it is interesting to remember the similar appearance of the bodies in the salivary glands in the insect. (4) A part of the appearance may be due to a cell product induced by the developing parasite.

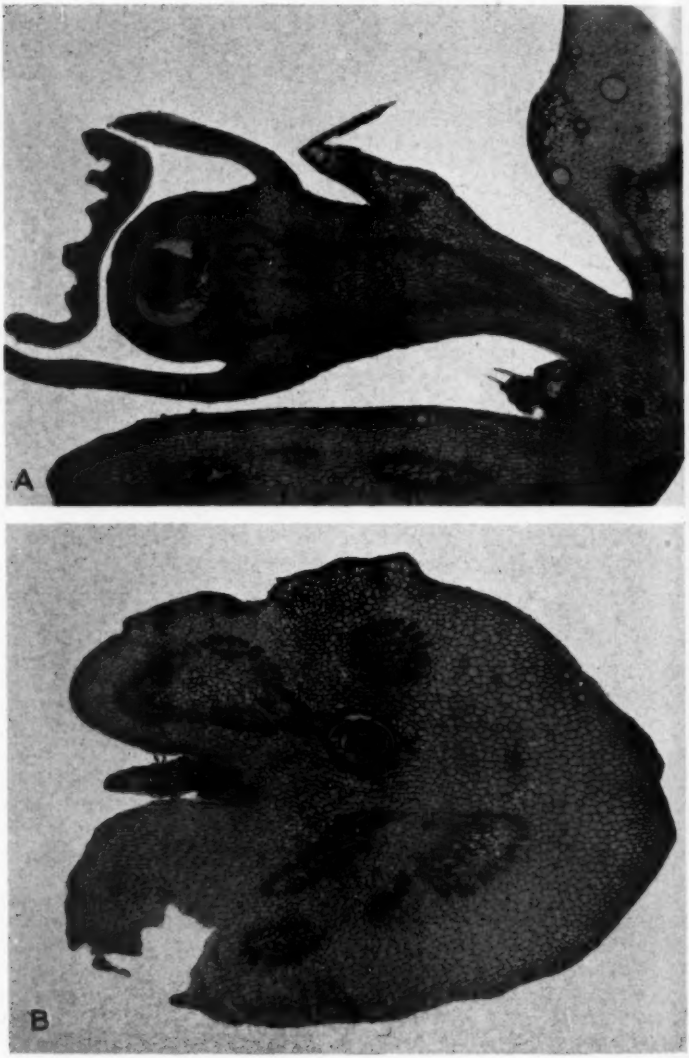

FIGURE 7.—A, Three feeding places of the cotton flea hopper on a bud of cotton, the plant having been preserved after the insects had fed on the bud for 24 hours; B, developing egg of *Psallus seriatus* in cotton bud, plant tissue otherwise normal

A STUDY OF FRESH MATERIAL OF THE COTTON FLEA HOPPER AND OF INFESTED COTTON

METHOD OF DISSECTION

During July, 1926, an attempt was made to get further information with regard to the exact method by which the cotton flea hopper injures the plant. It was found possible to take out entire the salivary glands of Psallus. The insects were placed on their sides in a watch glass and were completely covered with a thin layer of paraffin which covered the bottom of the glass and which had been thoroughly impregnated with powdered charcoal. It was easily possible then to cut away the chitinous body wall from both dorsum and side, exposing the alimentary canal and other organs.

The anterior part of the salivary gland is nearly transparent, with more opaque places which represent the nuclei of the cells. The posterior part of the salivary gland is usually white and opaque, and on dissection this appearance proves to be due to the secretion in its cavity, the walls of the gland being semitransparent, as in the case of the other half of the gland. Specimens have been found in which the entire gland was transparent. On the whole it is difficult to see these glands except against the black background of the dissecting dish.

TESTS FOR THE PRESENCE OF AMYLASE

In making tests for the presence of enzymes, the method used by Swingle (*11*) was followed, except that the starch solution was diluted to about 10 per cent. In the case of each test for the presence of amylase, a few drops of the mixture of starch colored with I–KI solution was placed in the center of each of two concave slides. The two glands from a single specimen of Psallus were removed, one placed on each slide and thoroughly torn to pieces. One slide was then heated until the drop of solution boiled, destroying the enzymes present. This slide was used as a control. Both were incubated at 35° C. for 48 hours. In a second test the glands were treated in a similar manner but without the addition of I–KI, and after incubation the resulting solution was tested for the presence of reducing sugars by means of Fluckiger's reagent. The tests were repeated twice, but in no cases was there any indication of the digestion of the starch. The tests may be taken as an indication of the absence of amylase, but the results should be checked in future by other methods, as the glands are extremely small. Amylase might, moreover, be secreted by one of the accessory salivary glands.

In another series of tests the insects were allowed to feed on a restricted area of the stem by means of the vials described in a preceding paragraph. Free-hand sections were cut through this part of the stem and tested for the location of the reducing sugars, glucose and fructose, by means of Fluckiger's reagent (*11, p. 215*). There was no distinguishable difference between the part of the cortex on which the insects had fed and the part which had been protected from them. Tests were made on material on which the insects had fed for 24, 48, and 72 hours, respectively. These tests seemed to show that injury to the plant was not due to a starch-digesting enzyme proceeding from the salivary glands.

EXAMINATION OF MATERIAL IN CELLS OF INFESTED PLANTS

In an effort to determine the nature of the material found in the cells of infested plants, free-hand sections were made of a number of lesions found in the field and also in cages where only one species of insect had been introduced. Affected plants taken from fields at Scott, Miss.; Tallulah, La.; and Port Lavaca, Tex.; and from cages from the two latter places, have been examined. In all cases an abundance of material similar to that noticed in the preserved plants has been found. Considerable difficulty has been experienced on account of the low visibility of the material in the cells of the fresh plants. The cells which are affected seem under the microscope to have a homogeneous grayish, opaque appearance and little or no detail can be made out. If I–KI solution, such as is used in testing for starch, is placed on the section, certain of these cells stain a homogeneous yellowish brown and others are seen to be packed full of nonstaining, round or oblong, glistening bodies of various sizes in different cells. Swellings on the midribs of leaves seem to afford the best material for a study of these objects in the cells. Their appearance does not differ, except as regards color, from that of preserved material previously described. Sections of stems which had swellings were treated with Fluckiger's reagent, and these showed a larger quantity of glucose and fructose in the area of the swelling than elsewhere. This was often concentrated in the cells which contained the granular material. Whether this is due to a peculiarity of this material or to the malformation of conducting tissue can not be determined at this time. The granular material in the cells has also been found in swellings from plants on which the tarnished plant bug (*Lygus pratensis* L.) had been caged.

EFFECTS OF THE HOPPER BITE ON PLANT CELLS

In order to study the immediate effects of the hopper bite, several vials containing the insects were fastened against the stems of protected plants and the insects allowed to feed through openings in the paper which replaced the bottom of the vial. A vial was removed on each successive day, and sections were cut out of the stem beneath it. These sections were examined both in water and in I–KI solution. The area on which the insects had fed contained many cells which were empty, their contents being replaced by air. In one case whole masses of cells were absent, but this was possibly due to injury during cutting. In all this material small oval or round bodies were found in the plant cells. These bodies were glistening and moved about freely in the cell or often in the water near a ruptured plant cell. They varied in number from one to a dozen or more per cell and seemed to increase in number with the increasing number of days on which the hopper had fed on the plant. It has not been possible to ascertain whether these bodies have a definite relation to the granular material which later appears in the cells of the plant. Sections have been examined of material on which the insects had fed from one to five days. No information regarding the origin of the granular material in the cells, however, has been gained by these examinations.

SUMMARY OF STUDIES WITH FRESH MATERIAL

Tests failed to reveal the presence of a starch-digesting enzyme in the salivary gland of Psallus, but the tests were not conclusive, and the accessory salivary glands were not tested.

Infested cotton plants from Mississippi, Louisiana, and Texas have been examined by means of free-hand sections.

In all cases material was found in the cells which resembled that found in the stained sections of plants from Port Lavaca, Tex.

Oval or round glistening bodies were found moving about in the cells of plants on which the Psallus had recently fed.

The observations suggest the possible presence of an organism which is transmitted by the cotton hopper but which does not penetrate far from the point of introduction.

This assumed organism may be transmitted by *Lygus pratensis* also.

The appearance of the cell inclusions studied suggests that of a myxomycete or slime mold, such as the one causing club root of cabbage.

GENERAL SUMMARY AND CONCLUSIONS

The primary purpose of this study is in the nature of foundation work for future studies. The anatomy and histology of the alimentary canal of the cotton flea hopper, *Psallus seriatus*, are given. The structure of the salivary glands and their accessory glands and ducts has been worked out, together with the structure of the mouth parts. The presence of bodies which may be parasites in the anterior part of the salivary gland is discussed.

The effect of the feeding of the cotton flea hopper on the plant cells has been described, and a study of the tissues of infested cotton has shown, in addition to the malformations of the cells, the presence of cell inclusions near the site of the puncture. In certain preparations these cell inclusions have the appearance of an invading or developing parasite and have been found in both fresh and preserved plant material.

The field experiments have shown that the inoculum or material injected by the cotton hopper does not spread far from the point of injury. The appearance of a systemic disturbance, sometimes observed in the infested fields, therefore, seems to be due to the multiplicity of bites, and the shedding of the hopper squares seems to be due to a bite near by.

The secondary purpose of the paper is to call attention to possible plant-disease transmission by Psallus and to present the evidence that has been gathered. Further work along this line must be done by the collaboration of a plant pathologist and an entomologist, and should include in addition a study of the feeding of other Miridae on cotton.

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TWO NEW SPECIES OF NODULAR WORMS (OESOPHAGOSTOMUM) PARASITIC IN THE INTESTINE OF DOMESTIC SWINE[1]

By BENJAMIN SCHWARTZ, *Senior Zoologist*, and JOSEPH E. ALICATA, *Junior Zoologist, Zoological Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

Before 1925 nodular worms occurring in the large intestine of domestic swine were considered a single species, *Oesophagostomum dentatum*. In 1925 Goodey [2] described a new species of this genus, collected in New Guinea from the intestine of a pig, presumably *Sus scrofa domestica*, and named it *O. longicaudum*. Shortly after the appearance of Goodey's paper, the senior author [3] reported the presence of *O. longicaudum* in domestic swine in the United States, the Philippine Islands, French Indo-China, and the Fiji Islands based on a study of specimens in the helminthological collections of the Bureau of Animal Industry.

A recent study of a small collection of Oesophagostomum from domestic swine obtained by E. W. Nighbert, of the Bureau of Animal Industry in Moultrie, Ga., at the request of the senior author, revealed the presence of *O. dentatum* and *O. longicaudum* and of two additional species of this genus heretofore undescribed. One of these species (for which the name *O. brevicaudum* is proposed) was present in fairly large numbers, whereas the other (for which the name *O. georgianum* is proposed) was represented by 6 specimens, of which 4 were males and 2 females, one of the latter being immature.

DESCRIPTION OF SPECIES

Oesophagostomum brevicaudum, new species.

The mouth opening is in the center of the mouth collar, which is surrounded by an external leaf crown composed of from 14 to 16 elements and an internal leaf crown composed of from 28 to 32 elements. (Fig. 1.) In 8 specimens in which the head was studied in frontal view the following numbers of elements in the leaf crowns were noted: 5 males showed 14, 15, 16, 16, and 16 elements in the external leaf crown and 28, 30, 32, 32, and 32 elements in the internal leaf crown, respectively; 2 females showed 14 elements in the external leaf crown and 28 elements in the internal leaf crown, and 1 female showed 16 elements in the external leaf crown and 32 elements in the internal leaf crown. The elements of the external and internal leaf crowns are triangular in shape; those of the internal leaf crown are located at the base of those of the external leaf crown. The elements of the latter project a little beyond the mouth opening. (Fig. 2.) The head bears four submedian papillae and two lateral papillae or amphids. The buccal capsule is small, its walls, as seen in optical section (fig. 2), having a morphology characteristic of this species and consisting of a more or less triangular apical portion and a more or less spherical basal portion; the two portions are separated by an indentation that is more pronounced on the outer than on the inner margin. The cuticle between the cephalic groove in front and the cervical

[1] Received for publication Nov. 13, 1929, issued March, 1930.

[2] GOODEY, T. THE ANATOMY OF OESOPHAGOSTOMUM DENTATUM (RUD.), A NEMATODE PARASITE OF THE PIG, WITH OBSERVATIONS ON THE STRUCTURE AND BIOLOGY OF THE FREE-LIVING LARVAE. Jour. Helminthol. 2 (1): 1–14, illus. 1924.

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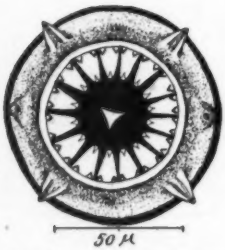

FIGURE 1.—*Oesophagostomum brevicaudum;* front view of head

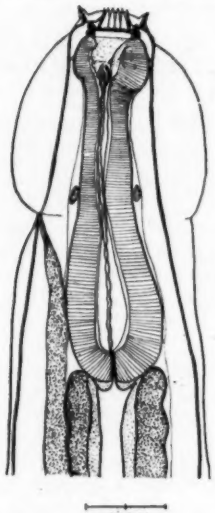

FIGURE 2.—*Oesophagostomum brevicaudum;* anterior portion of body. (Lateral view)

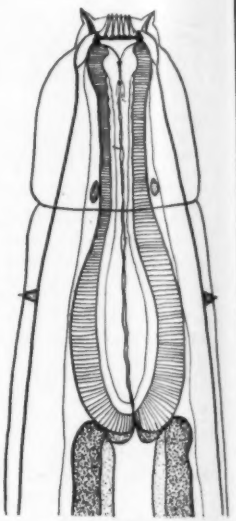

FIGURE 3.—*Oesophagostomum brevicaudum;* anterior view ofbody. (Ventral view)

FIGURE 4.—*Oesophagostomum brevicaudum;* male bursa. (Lateral view)

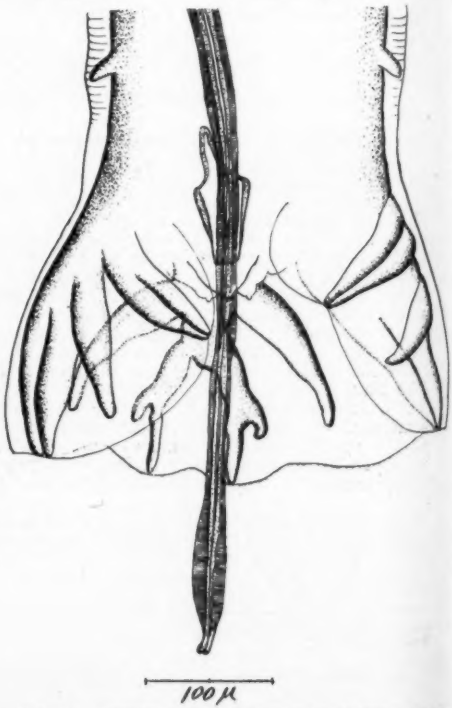

FIGURE 5.—*Oesophagostomum brevicaudum;* male bursa. (Ventra view)

groove behind is inflated. (Figs. 2 and 3.) The cervical groove is well developed ventrally and disappears dorsally. The cervical inflation of the cuticle extends to a point anterior to the middle of the esophagus. The esophagus, which is club shaped, has swollen sides at its beginning, and, as seen in lateral view, the dorsal wall of this swollen region is more developed than the corresponding ventral wall. (Figs. 2 and 12, A.) The inner wall of the swollen region contains a small toothlike protuberance on each side, projecting downward into the esophageal lumen. The outer margin of the cuticular lining of the esophagus is rounded into numerous knobs, replaced by a sinuous outline in some specimens. The excretory pore is immediately behind the cervical groove. The cervical papillae (fig. 3) are somewhat anterior to the last third of the esophagus.

Male.—According to observations of five specimens the variation in length is from 6.2 to 6.8 mm. and the range in maximum width is from 310μ to 449μ. The diameter of the body in the region of the prebursal papillae shows a variation of 173μ to 225μ. The esophagus is from 363μ to 449μ long by 124μ to 139μ in maximum width. The excretory pore is 171μ to 217μ from the beginning of the esophagus. The arrangement of the rays of the bursa (figs. 4 and 5) is similar to that of *Oesophagostomum dentatum* and *O. longicaudum*. The spicules are filiform, equal, and from 1.05 to 1.23 mm. long. The gubernaculum is from 98μ to 110μ long and from 37.5μ to 45μ in maximum width. The shapes of the gubernaculum and telamon (fig. 6) are similar to those described by Goodey for *O. dentatum*.

FIGURE 6.—*Oesophagostomum brevicaudum*: telamon and gubernaculum

Female.—According to measurements of five specimens the variation in size is from 6.4 mm. to 8.5 mm. in length and from 310μ to 450μ in maximum width. In the region of the vulva the diameter of the body varies from 108μ to 162μ and in the region of the anus it varies from 62μ to 70μ. The esophagus is from 434μ to 465μ long and from 120μ to 157μ in maximum width. The excretory pore is 186μ to 232μ from the beginning of the esophagus. The vulva, which is protuberant in some specimens, is 190μ to 225μ from the tip of the tail. The vagina (fig. 7) extends anteriorly and is from 218μ to 285μ long; the ovejector apparatus is from 165μ to 195μ long. The eggs are from 52.5μ to 67.5μ long by 30μ to 45μ wide. The tail is from 81μ to 120μ long, is directed dorsad and ends in a slender tip.

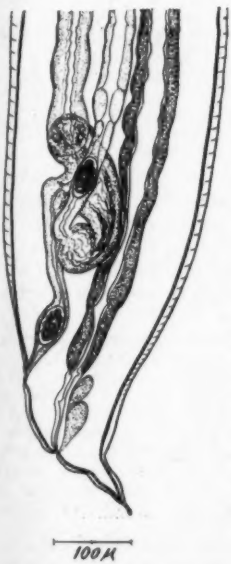

FIGURE 7.—*Oesophagostomum brevicaudum*; posterior end of female. (Lateral view)

Host.—*Sus scrofa domestica.*

Location.—Large intestine.

Locality.—Moultrie, Ga.

Type specimens (male and female).—Bureau of Animal Industry helminthological collections, United States National Museum No. 29038.

Paratypes.—United States Bureau of Animal Industry helminthological collections, United States National Museum No. 29039.

Oesophagostomum brevicaudum resembles *O. longicaudum* in the morphology of the esophagus and differs from the latter as well as from *O. dentatum* in many of the other specific characters. The principal differences are as follows: The walls of the buccal capsule in *O. brevicaudum*, as seen in optical section, present a characteristic appearance and differ from those of other species of Oesophagostomum parasitic in swine. (Fig. 12.) As seen in optical section (lateral view), the walls of *O. longicaudum* collected from swine in Georgia also show a distinct asymmetry, a character not noted by Goodey in his description of this species. Goodey's figure of the anterior view of this species shows symmetrical walls, diverging posteriorly. Aside from the relatively large

number of elements in the leaf crowns present in *O. brevicaudum* as compared with the number in *O. dentatum* and *O. longicaudum*, *O. brevicaudum* is readily recognized by the short female tail and the long vagina. The spicules in *O. brevicaudum* are considerably longer than those of *O. longicaudum* and are about the same length as those of *O. dentatum*.

Oesophagostomum georgianum, new species.

The head structures are similar to those of *Oesophagostomum dentatum.* (Fig. 8.) The mouth is surrounded by an external leaf crown consisting of 9 elements and

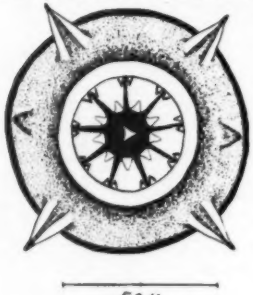

FIGURE 8.—*Oesophagostomum georgianum;* front view of head

an internal leaf crown of 18 elements, as in *O. dentatum* and in *O. longicaudum*. The esophagus is club-shaped, like that of *O. dentatum*.

Male.—The male is from 5.8 to 7.6 mm. long and from 388μ to 419μ in maximum width; the diameter of the body in the region of the prebursal papillae is from 232μ to 248μ. The excretory pore is immediately posterior to the cervical groove at a distance of 140μ to 186μ from the beginning of the esophagus. The esophagus is from 333μ to 377μ long and 109μ in maximum width. The width of the bursa when spread out is 310μ. The arrangement of the rays of the bursa (fig. 9) is similar to that shown in Goodey's figures[4] of *Oesophagostomum dentatum* and *O. longicaudum*. The spicules are equal, filiform, and from 1.015 to 1.131 mm. long. The gubernaculum (fig. 10) is from 115μ to 130μ long, or somewhat longer, and differs from that of other species of the genus found in swine in that the anterior narrow portion of this structure is twisted, the entire structure resembling an inverted capital "L" when viewed from the side (fig. 9); in *O. dentatum*, *O. longicaudum*, and *O. brevicaudum*, the anterior portion of the gubernaculum is straight, and, when viewed from the side, it appears as an elongated body (fig. 4) with an indentation which forms an obtuse angle.

FIGURE 9.—*Oesophagostomum georgianum;* male bursa. (Lateral view)

Female.—Of two specimens one was gravid. The mature female is 10 mm. long and 388μ in maximum width; the width of the body in the region of the vulva is 233μ and in the region of the anus, 93μ. The esophagus is 377μ long and 109μ in maximum width. The vulva (fig. 11) is 511μ from the tip of the tail; the tail is 217μ long and ends in a slender, terminal portion having a marked ventral curvature. The distance between the vulva and the anus is greater than the

[4] GOODEY, T. OESOPHAGOSTOMUM LONGICAUDUM N. SP. FROM THE PIG IN NEW GUINEA. Jour. Helminthol. 3 (1): 45-50, illus. 1925.

length of the tail. In the mature specimen this distance is about 300μ, and in the immature specimen it is 217μ. The vagina is almost transversely elongated and is 109μ long in both specimens. The length of the ovejector apparatus in the mature specimen is 202μ, and in the immature specimen it is 155μ.

Practically all the measurements of the immature specimen are smaller than those of the gravid specimen. The length of the former is 5 mm. and its maximum width is 263μ. Its esophagus is 325μ long and 78μ in maximum width. The vulva in this specimen is 395μ from the tip of the tail. The tail is 178μ long.

FIGURE 10.—*Oesophagostomum georgianum:* gubernaculum

Host.—*Sus scrofa domestica.*

Location.—Large intestine.

Locality.—Moultrie, Ga.

Type specimens (male and female).—Bureau of Animal Industry helminthological collection, United States National Museum No. 29064.

Paratypes.—Bureau of Animal Industry helminthological collection, United States National Museum No. 29065.

The heads of the four species of nodular worms are shown in Figure 12.

Oesophagostomum georgianum is very closely related to *O. dentatum*, differing from the latter in two important respects, namely, the shape of the gubernaculum and the shape of the female tail. As noted by Goodey, the gubernaculum in *O. dentatum* resembles a coal shovel or garden spade, with the handle and the blade about the same length. In *O. dentatum*, *O. longicaudum*, and *O. brevicaudum* the handle is straight, while in *O. georgianum*, it is distinctly twisted, as shown in Figure 10. In *O. georgianum* the handle is considerably shorter than the blade, whereas in *O. dentatum* these two parts are approximately equal. The female tail is longer in *O. dentatum* than in *O. georgianum*. The tail of the gravid female of the latter is 217μ long, whereas, according to Goodey, the length of the tail of *O. dentatum* is about 350μ in sexually mature forms. However, too much emphasis can not be placed, as a general rule, on the length of the female tail as a specific character in nematodes. Aside from differences in the length of the tail in the two species, an important difference in the shape of the tail has been noted. In this respect *O. georgianum* differs strikingly from *O. dentatum;* in the latter the female tail is straight and tapers to a point, whereas in *O. georgianum* the female tail is sinuous on its ventral surface and ends in a curved tip, the convexity of the curve being ventral and the terminal portion of the tail being directed dorsally. Another difference between *O. georgianum* and *O. dentatum* is the distance between the anus and the vulva as compared with the distance from the anus to the tip of the tail. In *O. dentatum* these two distances are approximately the

FIGURE 11.—*Oesophagostomum georgianum;* posterior end of female. Lateral view

same, the anus being equidistant from the vulva and the tip of the tail; in *O. georgianum* the distance between the tip of the tail and the anus is considerably smaller than that between the anus and the vulva.

Although the six specimens assigned to the species *Oesophagostomum georgianum* may perhaps represent aberrant forms of *O. dentatum*, it is believed that the differences between the forms which have been designated *O. georgianum* and *O. dentatum* warrant the recognition of the former as a distinct species. This view conforms to the usual custom

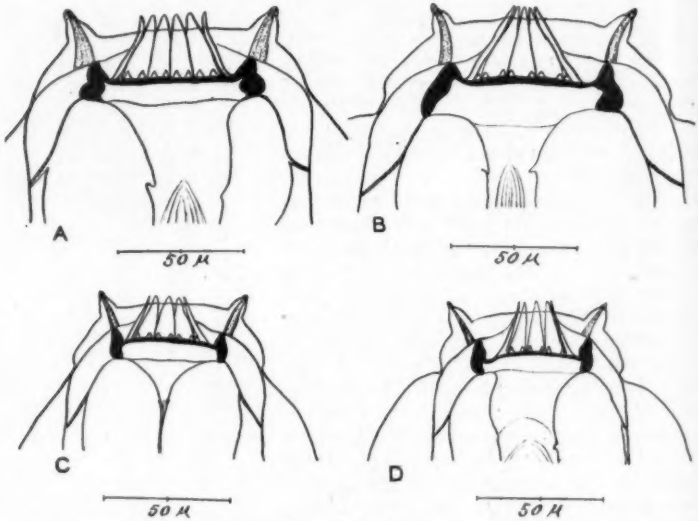

FIGURE 12.—A, Head of *Oesophagostomum brevicaudum*; B, head of *O. longicaudum*; C, head of *O. dentatum* D, head of *O. georgianum*

among helminthologists of creating new species in all cases in which the identity of closely related forms is doubtful.

SUMMARY

A morphological description is given of two new species of nodular worms (Oesophagostomum) recently found in the intestines of domestic swine. These species have been named *O. brevicaudum* and *O. georgianum* and, while the possibility has not been overlooked that the specimens herein assigned to the latter species may represent aberrant forms of *O. dentatum*, it is believed that observed morphological differences warrant the recognition of *O. georgianum* as a distinct species.

SELECTION CHARACTERS AS CORRELATED WITH PERCENTAGE OF SUCROSE, WEIGHT, AND SUCROSE CONTENT OF SUGAR BEETS[1]

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INTRODUCTION

The purpose of this paper is to summarize briefly the available information on the selection characters of sugar beets; to correlate these and other selection characters of the beet with its percentage of sucrose, its weight, and its sucrose content under carefully controlled conditions; and to present certain new data that bear on this subject. Partial correlation coefficients are presented with special reference to the improvement of beets that are poor in yield or quality but which may be desirable from other viewpoints (for example, disease resistance). The studies reported may thus be considered as in a sense preliminary to the work of breeding sugar beets for better adaptation to the various areas of the United States.

The literature on the selection characters of the sugar beet is comprehensive (*10*) [2] and generally instructive, though certain conclusions are misleading, owing to the material used or the methods of investigation. This is rather to be expected, since some of this work marks the beginning of agricultural science. The literature deals almost entirely with the percentage of sucrose in relation to various selection characters, thus disregarding the weight and the sucrose content. With few exceptions, the investigators have used practical breeding material, which is unsuitable, according to Oetken (*25*), because breeders have previously eliminated all the material below the mean weight and rooty, large-crowned, and otherwise undesirable beets. This practical breeding material has been taken largely from mass selections rather than individual lines. Investitors have not controlled the water intake or outgo of beets, which necessarily influences the percentage of sucrose, in the beet roots (*28*). In many instances the data are questionable because the analyses of the beets were delayed until after storage (*9*, *26*, *36*). Only the more recent workers have used statistical methods for expressing these relationships among various characters. These are probably some of the reasons why this mass of literature contains many contradictory statements.

MATERIAL

The material studied consisted of five lots of sugar beets which had been grown previously at the United States Sugar Plant Field Station, Salt Lake City, Utah. Each lot was considered representative of

[1] Received for publication June 26, 1929; issued March, 1930.

[2] Reference is made by number (italic) to "Literature cited," p. 544.

the material used in sugar-beet breeding work. The material in its final form contained 496 beets. Two beets were injured during cultivation, and two were diseased. Except for this elimination the material was complete. No beets were discarded because of extreme weight, percentage of sucrose content, or any other character, except as herein stated. Because of the disadvantage of incomplete material for statistical purposes, the writer made an effort to retain the material in a form as nearly complete as possible.

The five lots of material were selected so as to represent lines of sugar beets in different stages of breeding development. Lots 2 and 3 were produced by individual selection, lots 1 and 4 from mass selection, and lot 5 is a European élite strain. When arranged in descending sequence with respect to the more important characteristics of the beet, these lots have the following order: In average percentage of sucrose—3, 2, 4, 1, 5; as to purity of juice—2, 3, 4, 1, 5; as to yield of topped beets—5, 2, 4, 1, 3; as to sucrose content—5, 2, 4, 3, 1; as to recoverable sugar—2, 5, 3, 4, 1. Lot 3 had the highest average percentage of sucrose, while lot 2 had the highest average purity of juice (*44*). Lots 2 and 3 are of the sugar type and lot 5 of the yield type. Lots 4 and 5 showed marked variation as to foliage characteristics. Lot 5 was the most vigorous, as is indicated by its foliage characteristics and superior yield.

METEOROLOGICAL DATA

The climatic conditions for the crop of this particular year (1925) were very favorable. Not only were the beets on the experimental plot good, but in general all other fields showed large yields. Utah and Idaho during this season produced their largest recorded acre yields of recoverable sugar. The meteorological data for the growing season are given in Table 1. These data show that the season of 1925 was characterized by greater precipitation and higher relative humidity than the normal season.

TABLE 1.—*Meteorological data for Salt Lake City, Utah, April 1 to October 31, 1925*

[Normal data for the same locality are shown for comparison]

Month	Season of 1925								Normal season						
	Temperature			Precipitation	Relative humidity			Sunshine	Temperature			Precipitation	Relative humidity			Sunshine
	Maximum	Mean	Minimum		6 a. m.	Noon	6 p. m.		Maximum	Mean	Minimum		6 a. m.	Noon	6 p. m.	
	°F.	°F.	°F.	In.	P. ct.	P. ct.	P. ct.	Hrs.	°F.	°F.	°F.	In.	P. ct.	P. ct.	P. ct.	Hrs.
April	60.9	51.7	42.5	2.37	58.9	41.0	38.7	93.2	59.5	49.6	39.8	2.04	60	47	40	*83.8
May	74.0	63.3	52.6	1.90	52.4	31.6	30.2	345.7	68.5	57.8	47.1	1.99	57	36	35	300.8
June	75.8	65.6	55.3	2.80	59.5	39.3	35.2	302.8	79.3	67.4	55.5	.81	49	30	28	348.0
July	90.3	78.8	67.2	1.30	47.5	28.5	28.6	362.1	88.2	75.9	63.7	.55	46	30	25	362.1
August	83.8	73.0	62.2	1.65	53.0	30.5	29.3	303.5	86.5	74.6	62.6	.83	47	30	27	324.4
September	73.7	63.6	53.4	.29	58.6	35.1	33.0	276.8	76.2	64.5	52.8	.94	50	34	32	284.2
October	60.8	51.9	43.0	.65	57.3	37.8	41.8	230.5	62.6	52.3	42.1	1.52	59	43	46	230.5
Average	74.2	64.0	53.7	.....	55.3	34.8	33.8	.....	74.4	63.2	51.9	.....	52.6	35.7	33.3	.....
Total	.....	.....	.....	10.96	.....	.....	.....	1,914.6	.....	.....	.....	8.68	.....	.....	.....	1,933.8

* Only one-third of the sunshine for April is considered, because the plants did not appear above the ground until Apr. 20.

GENERAL METHODS

The five lots of seed were planted April 9, 1925, in five parallel rows on a plot of land which was selected because of its past record of uniformity in production. Soon after thinning, 100 consecutive beets from each row were given identification numbers that remained with them throughout the entire investigation. The soil, climate, and cultural conditions of these beets were very uniform, as the material was taken from relatively short sections at the middle of long rows.

During the last few years sugar-beet breeders have become increasingly aware of the need for measuring all sugar-beet characters in definite units. All but four characters herein given were measured in standard units or else compared with a standard.[3] The work was carefully planned so that all measurements could be made on the same individual beets. The position of the beet in the soil and the foliage characters were determined by measurements in the field. As far as possible, the data were collected just before harvest; consequently the quality and quantity of the characters correspond very closely to the percentages of sucrose or to other valuable characteristics of the beets as determined at the time of analysis.

The procedure that made possible the accomplishment of the analysis before any considerable changes had occurred was as follows. At harvest the soil was removed from one side of each beet and the root was given its identification number by means of a permanent spiral tag (*27*) before it was removed from the ground. The leaves were trimmed off, leaving portions of the petioles about 5 mm. long on the crown. The leaves from each beet were weighed, and the beet was immediately placed in a covered can. As soon as the harvest had been completed the roots were cleaned, weighed to within 5 gm. of accuracy, and then analyzed. The percentage of sucrose (parts of sucrose per 100 parts of the total substance) in the beet was determined by direct polarization. The sucrose content (actual weight of sucrose) of the beet was determined by multiplying the weight of the root by its percentage of sucrose. The apparent purity was calculated from the percentage of sucrose in the juice and the total solids in the juice as determined by the refractometer. The other characteristics of the beet root were recorded soon after the chemical analysis. All beets were carefully protected against changes (*28*) during the entire period of harvest investigation. There were no beets that showed appreciable change of weight during this period, and therefore no corrections in the percentage of sucrose for water intake or outgo were necessary. The quantity of sugar loss due to other causes would have been negligible because of special care of the beets and their immediate analysis.

In all, enough measurements were made on this material for the calculation of about 3,000 correlation coefficients. From these data about 500 correlation coefficients have been calculated for the relationships between the valuable qualities of the beet root and the morphological, anatomical, physical, and chemical characteristics of the beet plant. The more valuable quantities of the beet root are its weight, its percentage of sucrose, its sucrose content, and its purity. The final criterion for evaluating the beet (the recoverable sugar per unit cost) is partly dependent upon these qualities.

[3] The measurements of all characters were made by Vera Sanders and the writer.

The data for all lots were grouped, and the total correlation coefficients were calculated for the entire material. One disadvantage of this grouping is that a definite trend of relationship in one line may balance an equally definite reverse trend of relationship in a second line. To overcome this disadvantage the data for each line were kept separate and wherever a balancing of relationship between lines was noted correlation cofficients were calculated for the separate lines.

The data were classified and scatter diagrams were made for all characters. Sheppard's correction was made for grouping, and the correlation coefficients were calculated by the product-moment method. The biserial correlation coefficient was not used except for characters 7 and 11. The probable errors for the coefficients were calculated and read from Pearson's Table 7 (*30*). Partial and multiple correlation coefficients were calculated for the principal characters.

EXPERIMENTAL METHODS AND RESULTS

The total correlation coefficients for the relationships between the percentage of sucrose, the weight, and the sucrose content with 47 other characters of the beet plant for the entire material are given in Table 2. Table 3 gives partial correlation coefficients for some of these relationships.

The relationships among the various characters of the beet will be considered in the order given in Table 2. For convenience in referring to these characters and coefficients of correlation, it is necessary to indicate the characters by the numbers given in column 1 of Table 2. These numbers will also be used as correlation subscripts.

PERCENTAGE OF SUCROSE AND WEIGHT

CHARACTER 1.—The relationship between the percentage of sucrose in the root (character 1; sometimes erroneously designated as sugar content) and the weight of the beet root (character 2) is of major importance because it deals with the two fundamental characteristics of the sugar-beet root. This relationship has been studied and reported by many investigators. These authors differ in opinion as to the extent and existence of this relationship. Remy (*37*) summarizes the results of work covering seven years on 28 sorts, as follows: 17.44, 16.98, and 16.30 per cent of sucrose, corresponding to 331, 348, and 357 doppelzentners (220 pounds) yield of beets, respectively. These results indicate a negative relation between the percentage of sucrose and the weight of beet. Oetken's extensive work (*25*) shows both negative and positive relations between these two characters. Rümker (*38*) in his examination of 100,000 beets as to percentage of sucrose and weight found a negative relation between these characters, and states that with each 0.29 decrease in the percentage of sucrose there was an increase of 100 gm. in weight. Novotný (*24*) found that from 1892 to 1900 the percentage of sucrose decreased from 0.39 to 0.19 per cent for each 100 gm. of increased weight. Oetken (*25*) found that for certain lines the decrease in the percentage of sucrose was only 0.1 per cent per 100 gm. of the weight of beet. Fruwirth and others (*10*) state that the concept of a negative correlation between the percentage of sugar and the weight of beet should be dismissed and that these characters vary independently of each other.

TABLE 2.—*Total correlation coefficients of the sugar-beet characteristics*

[Descriptions of the various characters (numbered 1 to 50 in column 1) together with details of their measurement are given in correspondingly numbered paragraphs in the text]

No. and character	Coefficient of correlation with—		
	Percentage of sucrose	Weight	Sucrose content
1. Percentage of sucrose		−0.40±0.03	−0.23±0.03
2. Weight	−0.40±0.03		+.95 ±.00
3. Sugar content	−.23 ±.03	+.95 ±.00	
4. Angle of shoulder (obtuseness)	−.32 ±.03	±.27 ±.03	+.25 ±.03
5. Area of cross section of petiole	−.37 ±.03	+.34 ±.03	+.31 ±.03
6. Area of leaf	−.33 ±.03	+.49 ±.02	+.46 ±.02
7. Blonski selector	+.23	−.25	−.22
8. Color of flesh	−.23 ±.03	+.35 ±.03	+.31 ±.03
9. Color of leaf	+.06 ±.03	+.25 ±.03	+.30 ±.03
10. Conductivity	−.22 ±.03	+.28 ±.03	+.22 ±.03
11. Density	+.51	−.33	−.19
12. Density of foliage	−.21 ±.03	+.47 ±.02	+.66 ±.02
13. Depth of petiole groove	−.14 ±.03	+.30 ±.03	+.28 ±.03
14. Depth of root suture	+.05 ±.03	+.05 ±.03	+.08 ±.03
15. Diameter of crown	−.36 ±.03	+.86 ±.01	+.86 ±.01
16. Dry substance in root	+.81 ±.01	−.30 ±.03	−.15 ±.03
17. Extension of shoulder	+.29 ±.03	−.10 ±.03	−.06 ±.03
18. Foliage area	−.35 ±.03	+.60 ±.02	+.58 ±.02
19. Habit (prostrate foliage)	+.22 ±.03	−.05 ±.03	−.02 ±.03
20. Hardness of root tissue	+.43 ±.02	−.26 ±.03	−.19 ±.03
21. Height of crown	−.46 ±.02	+.77 ±.01	+.74 ±.01
22. Leaf density	+.37 ±.03	−.64 ±.02	−.64 ±.02
23. Length of leaf	−.32 ±.03	+.38 ±.03	+.41 ±.03
24. Length of root	−.14 ±.03	+.75 ±.01	+.64 ±.02
25. Margin of leaf	−.19 ±.03	+.17 ±.03	+.16 ±.03
26. Number of leaves	−.22 ±.03	+.54 ±.02	+.52 ±.02
27. Number of vascular-bundle rings	−.01 ±.03	+.36 ±.03	+.39 ±.03
28. Position in the soil	−.39 ±.03	+.70 ±.02	+.69 ±.02
29. Purity (apparent)	+.38 ±.03	−.17 ±.03	−.10 ±.03
30. Refractometer reading	+.81 ±.01	−.30 ±.03	−.15 ±.03
31. Relative depth of petiole groove	+.00 ±.03	+.13 ±.03	+.16 ±.03
32. Relative flatness of root	+.05 ±.03	+.03 ±.03	+.04 ±.03
33. Relative height of foliage	−.16 ±.03	+.10 ±.03	+.02 ±.03
34. Relative length of blade	−.07 ±.03	−.03 ±.03	−.05 ±.03
35. Relative length of leaf	−.03 ±.03	−.09 ±.03	−.10 ±.03
36. Relative length of petiole	+.07 ±.03	−.10 ±.03	−.10 ±.03
37. Relative length of root	+.14 ±.03	−.19 ±.03	−.20 ±.03
38. Relative space per beet	−.02 ±.03	+.20 ±.03	+.21 ±.03
39. Ring density	+.31 ±.03	−.68 ±.02	−.67 ±.02
40. Rootiness of beet	−.17 ±.03	+.12 ±.03	+.11 ±.03
41. Roughness of skin	+.36 ±.03	+.01 ±.03	+.08 ±.03
42. Rugosity of leaf surface	−.17 ±.03	+.16 ±.03	+.18 ±.03
43. Shade of leaf	+.24 ±.03	+.10 ±.03	+.17 ±.03
44. Sugar marks	+.17 ±.03	+.12 ±.03	+.16 ±.03
45. Thickness of leaf	−.14 ±.03	+.17 ±.03	+.16 ±.03
46. Turn of root suture	+.03 ±.03	−.05 ±.03	−.04 ±.03
47. Weight of foliage	−.33 ±.03	+.56 ±.02	+.54 ±.02
48. Width of leaf	−.23 ±.03	+.41 ±.03	+.43 ±.02
49. Width of petiole groove	−.25 ±.03	+.23 ±.03	+.20 ±.03
50. Width of root suture	−.10 ±.03	+.02 ±.03	−.00 ±.03

Table 2 shows a negative correlation coefficient (-0.40 ± 0.03) between the percentage of sucrose and the weight of beet for all the material investigated. For this relationship the separate lines gave the following values of r: 1, -0.27 ± 0.06; 2, -0.52 ± 0.05; 3, -0.33 ± 0.06; 4, -0.53 ± 0.05; and 5, -0.48 ± 0.06. These coefficients are quite different for the different individual lines, yet they are of the same sign. As would be expected, the correlation coefficients are less for the more homogeneous lines. Since Velasco (*45*) states that the leaves and the weight of the beet are functions of climate and conditions, it might be asked are these relationships between the percentage of sucrose and the weight of beet controlled by these factors? So far as climate is concerned, it must have been uniform over this small experimental plot. The available space for each beet in the field was

different, but this experimental difficulty was overcome by calculating the partial correlation coefficients. As the partial correlation coefficient for the percentage of sucrose with the weight constant for relative space ($r_{12 \cdot 38} = -0.4036 \pm 0.03$) is practically identical with the total correlation coefficient ($r_{12} = -0.40 \pm 0.03$), there is no indication that the relationship between the characters 1 and 2 is affected by the available space in the field. Even though available space does not influence this relationship, it has a direct effect upon character 2, as will be pointed out later. The partial correlation coefficient of the percentage of sucrose with the weight constant for sucrose content of the beet root was calculated as -0.6271 ± 0.02, indicating that this relationship between characters 1 and 2 is influenced by the sucrose content of the root. These results with the majority of investigations and the usual field and factory results in all sugar-beet countries show that there is in general a negative relation between the percentage of sucrose in the beet root and its weight. This relationship has a physiological explanation and should for the present be spoken of as physiological correlation. It is not possible from the data now available to ascertain what part, if any, of this relationship is hereditary in nature, or whether there is any true correlation between characters 1 and 2.

PERCENTAGE OF SUCROSE AND SUCROSE CONTENT

CHARACTER 2.—The relationship between the percentage of sucrose in the beet root (character 1) and the sucrose content of the beet root (actual quantity of sucrose, character 3) is of importance because 3 approaches the criterion for evaluating sugar beets. Janasz (*12*), in comparing various sugar-beet races, records figures for characters 1 and 3. Plahn (*33*) and Mass (*17*) state that character 1 decreased whereas character 3 increased. The total correlation coefficient for the relationship between characters 1 and 3 is given in Table 2 as -0.23 ± 0.03. The separate lines gave the following total correlation coefficients for this relationship: 1, -0.14 ± 0.07; 2, -0.41 ± 0.06; 3, -0.24 ± 0.07; 4, -0.24 ± 0.07; and 5, -0.27 ± 0.07. It was thought that the sucrose content (character 3) should increase with the increase of percentage of sucrose in the root (character 1), but these coefficients signify a negative relation. From this it would appear that beets of the highest sucrose content would be bred by selecting those of low percentage of sucrose. The inconsistency of this was apparent, and the first step toward clarifying the situation was to determine the partial correlation coefficient $r_{13 \cdot 2}$. The value for this partial correlation of character 1 with character 3 constant for character 2 was calculated as $+0.52 \pm 0.02$. (Table 3.) This coefficient is significant, and shows that the sucrose content does increase as the percentage of sucrose increases so long as the beets are of uniform weight. This explains why earlier investigators reported a negative relation between characters 1 and 3, instead of the positive relationship which exists, and shows the inconsistency of selecting low percentage sugar beets for high sucrose content. As these results show that the percentage of sucrose is positively correlated with the sucrose content when the factor for root weight is eliminated, the increase of the percentage of sucrose in the beet is of primary importance to sugar-beet breeding. It may be added that the first and second order

coefficients of correlation $r_{13\cdot38} = -0.23 \pm 0.03$ and $r_{13\cdot38\,2} = +0.52 \pm 0.02$ are the same as the total and first order coefficients $r_{13} = -0.23 \pm 0.03$ and $r_{13\cdot2} = +0.52 \pm 0.02$, indicating that available space in the field does not influence the relation between characters 1 and 3.

TABLE 3.—*Partial correlation coefficients of sugar-beet characters*

Selection character No.	Characters of column 1 correlated with—				Selection character No.	Characters of column 1 correlated with—			
	Percentage of sucrose, constant for weight	Weight constant for percentage of sucrose	Sucrose content, constant for percentage of sucrose	Sucrose content, constant for weight		Percentage of sucrose, constant for weight	Weight constant for percentage of sucrose	Sucrose content, constant for percentage of sucrose	Sucrose content, constant for weight
1	2	3	4	5	1	2	3	4	5
1				+0.52	22	+0.16	−0.58	−0.61	−0.13
2			+0.96		23	−.19	+.29	+.37	+.17
3	+0.52	+0.96			24	+.26	+.76	+.63	−.29
4	−.25	+.16	+.19	−.02	25	−.14	+.10	+.12	−.01
5	−.26	+.23	+.25	−.04	26	−.00	+.67	+.51	+.00
6	−.17	+.34	+.42	−.02	27	+.16	+.39	+.40	+.12
7	+.15	−.18	−.18	+.06	28	−.17	+.64	+.67	+.11
8	−.10	+.29	+.27	−.08	29	+.35	−.02	−.01	+.20
9	+.18	+.30	+.32	+.21	30	+.79	+.04	+.06	+.42
10	−.12	+.21	+.18	−.15	38	+.06	+.21	+.21	+.07
11	+.44	−.16	−.09	+.42	39	+.06	−.64	−.65	−.10
13	−.02	+.27	+.26	−.06	41	+.40	+.18	+.18	+.22
15	−.03	+.72	+.86	+.27	42	−.12	+.10	+.15	+.09
16	+.79	+.04	+.06	+.45	43	+.31	+.21	+.24	+.24
17	+.27	+.02	+.01	+.11	44	+.13	+.21	+.21	+.15
18	−.15	+.54	+.55	+.04	45	−.08	+.13	+.13	−.01
20	+.37	−.11	−.10	+.19	48	−.08	+.36	+.40	+.14
21	−.28	+.72	+.73	+.04	49	−.18	+.15	+.15	−.06

WEIGHT AND SUCROSE CONTENT

CHARACTER 3.—The relationship between the weight of the beet root (character 2) and the sucrose content of the beet root (character 3) is of great significance and has been seriously neglected by sugar-beet breeders. Janasz (*12*) published three figures indicating a positive relation between characters 2 and 3. Mass (*17*) showed that as the weight of feed beets increased their sugar content increased. Pritchard (*35*) published the first statistics expressing the relationship between characters 2 and 3 as $+0.92 \pm 0.0016$. Table 2 gives the total correlation coefficient for this relationship as $+0.95 \pm 0.00$. For this relationship the separate lines gave the following values for the total correlation coefficients: 1, $+0.98 \pm 0.01$; 2, $+0.98 \pm 0.01$; 3, $+0.94 \pm 0.01$; 4, $+0.98 \pm 0.01$; and 5, $+0.96 \pm 0.01$. The following partial correlation coefficients $r_{23\cdot38} = +0.95 \pm 0.00$, $r_{23\cdot1} = +0.96 \pm 0.00$, and $r_{23\cdot38\,1} = +0.96 \pm 0.00$ show that this relationship is not seriously influenced by either character 1 or character 38. All of these coefficients are significant and their values show a very strong relationship between characters 2 and 3. It is at once apparent that any gain in the weight of the beet will be accompanied by a corresponding increase in the sucrose content. In view of the fact that the sucrose content of the beet is directly proportional to the yield of sugar within certain minor limitations as has been noted, the sugar-beet breeder should give the weight of the root more consideration than has been done in the past.

PERCENTAGE OF SUCROSE WEIGHT AND SUCROSE CONTENT AS RELATED TO OTHER SELECTION CHARACTERS

The methods used in measuring each sugar-beet characteristic for all individual beets and in collecting the data for the correlations will be briefly stated. All of the typical relationships will be considered individually. The total correlations describing all relations for the entire material are presented in Table 2. Note will be made of any total correlation for the individual lines and the means of the individual lines that vary widely from those given in Table 2.

CHARACTER 4.—The angle which is formed by the intersection of the crown and root body surfaces was referred to in Table 2 as the "angle of shoulder." This angle was measured by a protractor. The coefficients $r_{14} = -0.32 \pm 0.03$ (Table 2) and $r_{14 \cdot 2} = -0.25$ (Table 3) show that beets with the more obtuse shoulder angles generally have a lower percentage of sucrose, or the correlative, that beets with more acute shoulder angles generally have a higher percentage of sucrose. Yet, the values for $r_{24} = +0.27 \pm 0.03$ and $r_{34} = +0.25 \pm 0.03$ indicate that beets having the more obtuse shoulder angles are larger and contain more sucrose. The partial coefficients $r_{24 \cdot 1} = +0.16$ and $r_{34 \cdot 1} = +0.19$ support these conclusions even after the relations with character 1 have been eliminated. The partial coefficient $r_{34 \cdot 1} = +0.19$ is significant, whereas the partial coefficient $r_{34 \cdot 2} = -0.02$ is not significant. This indicates that the larger sucrose content of beets with obtuse shoulder angles is essentially due to the larger weight of this type of beet root. Similar relations existed for the individual lines, but they were of irregular magnitude.

CHARACTER 5.—The area of the cross section of the sugar-beet petiole, aside from individual-plant differences, varies with the age and size of the leaf, the seasonal and field conditions, and the particular time of the grand period of growth in which the petiole was produced; thus, the first and last petioles produced by the beet are small. Finally the cross-section area of any beet petiole increases in the proximity of the leaf blade and the root crown. To avoid these and other difficulties, a certain leaf on each beet was selected for study. This leaf was termed by the writer the type leaf. The length of its leaf blade when near its height of vigor, growth, and development was 0.7 that of the longest leaf blades on the particular plant and specially characteristic of the plant. At harvest the petiole of the type leaf was sectioned with a razor at the point of minimum area and an ink stamp of its area recorded. The areas of these stamps were later determined by a planimeter and expressed in square millimeters. The correlation coefficients for character 5 with characters 1, 2, and 3 are similar to those for character 4 (Tables 2 and 3) and will permit of a like analysis with similar conclusions. (See character 4.)

CHARACTER 6.—Corenwinder and Contamine (*6*) were of the opinion that large leaves indicate a high percentage of sucrose. Vychinski (*48*) states that beets grown in the border row have large leaves and low percentages of sucrose. Kajanus (*13*) notes that large beets have large leaves, while Knauer and Doerstling (*14*) and Doerstling (*7*) prefer an average-sized leaf. Kneifel (*15*) and Marek (*16*) state that the size of the leaf varies throughout the season and with various types of soil. Investigators who have studied the total leaf surface of beets are mentioned under character 18. The type-leaf blade of each beet was photographed at harvest and its area measured as

square centimeters by a planimeter. The correlative coefficients for character 6 are similar to those for characters 4 and 5 (Tables 2 and 3) and will permit of like conclusions. (See character 4.)

CHARACTER 7.—Blonski (*1*) noted that beets having high percentages of sucrose were long in proportion to their diameter and he devised the Blonski selector for eliminating undesirable beets. Briem (*3*) criticized Blonski and stated that beets having a high percentage of sucrose may be either long or short. A selector was constructed by the writer according to Blonski's directions and each beet tested. Even though there are irregularities, as noted by Briem, the results in Tables 2 and 3 indicate a positive correlation between characters 1 and 7, as was first noted by Blonski.

CHARACTER 8.—The color of the beet flesh was determined by the writer from a sample located midway between the center and periphery of a middle cross section of the beet body. The following five colors illustrated by Ridgway [4] were recognized: 29″ g, 26″ g, white pallid-neutral gray, 20″ g, and white. In general, all beets were white, there being no deeply colored individuals, as spoken of by Munerati (*21*). Beets with even these very slight flesh colors tend toward a lower percentage of sucrose but a higher weight and sucrose content.

CHARACTER 9.—Some investigators noted increased percentage of sugar with dark-green foliage, others noted the opposite relation, and still others noted no relation. These varying opinions are due in part to the fact that the color of the sugar-beet leaf varies with age, light exposure, moisture, soil nutrients, and the season of the year. These difficulties were avoided by taking a sample from a particular locality on the type leaf. This sample was compared with a chart of standards distinguishing 20 different hues of green. These colors were made particularly for sugar-beet foliage, and range from Ridgway's 26‴ m to his lighter green 26 i. The total correlation coefficients of Table 2 show that character 9 is negligibly related to character 1, but positively related with characters 2 and 3. Late-maturing beets retain their dark-green foliage longer in the season. As these beets are usually large, it was possible to eliminate the error due to late-maturing beets by keeping the weight of the beets constant. This partial correlation of characters 1 and 9 constant for $2(r_{19\cdot2} = +0.18 \pm 0.03)$ is positive, and suggests a slight relation between the percentage of sucrose and the dark-colored foliage. It should be noted that the partial coefficients for character 9 with characters 1, 2, and 3 are all positive and significant. (Table 3.) This unique association shows that the percentage of sucrose, the weight, and the sucrose content of the sugar-beet root increase with the dark-green color of the foliage. Breeders should scrutinize this character in the improvement of sugar beets.

CHARACTER 10.—Conductivity was measured by inserting two platinized knife blades into the beet tissue at a definite point (halfway between the fleshy and suture sides and one-third the distance from the crown to the tip of the beet). In general, a lower conductivity was noted at the points of higher concentration of sucrose in the individual beet. This agrees with the work of Colin and Grandsire (*5*), though they worked on the extracted juice. The total and partial correlations for all the material and the individual lines showed

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

negative relation between characters 1 and 10, but positive relations between 2 and 10 and between 3 and 10.

CHARACTER 11.—The relation between the density of the beet and its percentage of sucrose has been studied by many writers. The beets were divided into two groups, "sinkers" and "floaters," by submerging a piece of tissue, as previously outlined by the writer (*29*), in a salt solution. The coefficients in Table 2 show that density is positively correlated with the percentage of sucrose, but negatively related to the weight and the sucrose content of the beet. These negative relations for character 11 with characters 2 and 3 were reduced and became less significant as the influence of character 1 was eliminated. The value of this relationship between density and the percentage of sucrose increased for the individual lines and amounted in one case to $+0.74$. This was undoubtedly due to the fact that the beets of pure lines are more uniform than the beets of heterogeneous lots in structure and in content of gases and salts—the characteristics that interfere with the density and the percentage of sucrose relationships. For a more complete analysis of the coefficients, see the paragraph relating to character 16, wherein a similar set of relationships is described.

CHARACTER 12.—The density of the foliage was estimated during September. It was noticed that this factor changed with the different periods of beet development and the changing external conditions. Owing to these and other difficulties (see character 22), the relations between density of foliage and other characters are questionable.

CHARACTER 13.—Pritchard (*35*) found a slight positive correlation between percentage of sucrose and depth of petiole groove. From the stamps of the petiole cross sections, made as described under character 5, the depths of the petiole grooves were accurately measured in millimeters. The total coefficients $r_{1\ 13}$ of Table 2 indicate a negative relation between characters 1 and 13. The partial correlation coefficient ($r_{1\ 13.2} = -0.02 \pm 0.03$) shows that there was no association of character 1 with character 13 if the weight of the beet was held constant. As expected, the depth of the petiole groove increases with the weight and the sucrose content of beets. (Tables 2 and 3.)

CHARACTER 14.—A deep suture on the root has associated with a high percentage of sucrose. In this connection the beet in the time of Achard had no well-defined suture. The depth of the suture was estimated by comparison with its width and should be accepted with caution. The correlation coefficients for character 14, given in Table 2, are not significant.

CHARACTER 15.—Beets with small crowns usually have the higher percentages of sucrose. The diameter of the crown was accurately measured with calipers at the point of the first leaf scar. The total coefficients of Table 2 indicate that characters 2 and 3 increased with character 15, whereas character 1 decreased. The partial coefficients of Table 3 show definitely that the percentage of sucrose was not significantly correlated with the diameter of the beet crown and that beets with large crowns were large and high in sucrose content. The sucrose content is significantly correlated with diameter of crown regardless of whether either character 1 or 2 was held constant.

CHARACTER 16.—It is generally conceded that dry substance is positively correlated with the percentage of sucrose. The dry substance in the beet root was determined by the refractometer. The total

and partial coefficients of Tables 2 and 3 show that the percentage of sucrose is significantly and positively correlated with dry substance. The total correlation coefficients of Table 2 indicate that the weight and the sucrose content are negatively correlated with dry substance. These negative relations are only apparent and are due to the strong positive relation between characters 1 and 16. The partial coefficients $r_{2\,16\cdot1} = +0.04$ and $r_{3\,16\cdot1} = +0.06$ of Table 3 show that the weight and the sucrose content are not significantly associated with dry substance if the factor for the percentage of sucrose is held constant. The significant coefficient $r_{3\,16\cdot2} = +0.42$ is due to the factor for percentage of sucrose not being eliminated.

CHARACTER 17.—The maximum extension of the surface of the beet beyond the first leaf scar was termed the extension of the shoulder. The percentage of sucrose increases with the extension of the shoulder, but there is no significant relation between character 17 and characters 2 and 3. The relations between characters 17 and 1 were very different for the individual lines, ranging from +0.17 to +0.40. However, the lines with the highest mean percentage of sucrose always had the largest mean extension of shoulder. Although the partial coefficients for character 17 are not so significant as those for character 16 (Table 3), similar conclusions should be drawn. (See character 16.)

CHARACTER 18.—The areas of beet leaves have been variously measured or estimated by several writers. Mass (*17*) found no relation between the leaf area and the percentage of sucrose. Munerati and his coworkers (*23*) found that defoliation of beets caused a decreased percentage of sucrose. Corenwinder and Contamine (*6*) claimed positive relation between characters 1 and 18, but Vychinski (*48*) questioned this. The total leaf surface of the beet during its entire vegetative period was in the present study termed the "foliage area." So far as the writer knows, this is the first attempt that has been made to determine the total foliage area produced by a beet. After planimeter measurements had been made of the total leaf area of beets during three different years it was found that the foliage area of a beet plant could be estimated by multiplying the area of the type leaf (character 5) by the total number of leaves produced during the vegetative period (character 26). The total correlation coefficients in Table 2 show that an increase of foliage area is accompanied by decreased percentage of sucrose, increased weight, and increased sucrose content. The partial correlation of the percentage of sucrose (character 1) with the foliage area (character 18) constant for the weight (character 2) gives a coefficient that is still significant ($r_{1\,18\cdot2} = -0.15 \pm 0.03$). Finally, the coefficient $r_{1\,18\cdot23} = -0.21 \pm 0.03$ shows that characters 1 and 18 are negatively correlated after the factors of weight and of sucrose content are eliminated. It was hoped to establish positive net relation between characters 1 and 18, but so far as the other factors have been considered the reverse is true; i. e., the percentage of sucrose increases with the decrease of foliage area. All the total and partial correlation coefficients show that the weight of the beet increases directly with the foliage area. The following coefficients—$r_{3\,18} = +0.58 \pm 0.02$, $r_{3\,18\cdot1} = +0.55 \pm 0.02$, and $r_{3\,18\cdot12} = +0.15 \pm 0.03$—show that the sucrose content increases with the foliage area.

CHARACTER 19.—Earlier investigators, with few exceptions, have noted an increased percentage of sucrose in the prostrate-foliage type of beet. The description prostrate-foliage habit was used to describe the angle that the leaf makes with the perpendicular. This angle is smaller for the younger leaves and increases with the age of the leaf. Leaf maturity, excessive transpiration (due to drought, high temperature, wind, dry soil, etc.), a lack of phosphorus, and hereditary characteristics are the more important causes of the prostrate habit. Owing to these disturbing factors, the type leaf was examined during the same period of the day and under as nearly uniform conditions as possible. Table 2 shows that character 19 is positively related to character 1 but not related to either character 2 or 3.

CHARACTER 20.—According to Geschwind (*11*), De Varies and Stoklasa found that lignified beet contains a smaller percentage of sucrose. Munerati (*20*) states that woody beets are not necessarily the bolting strains. Schribaux (*41*) states that the flesh of sugar beets has become firm and harder with the breeding of beets of increased percentage of sucrose. He states that Pelletier determined the value of beets by the force required to push a punch into the tissue. The hardness of the beet tissue was determined by a self-recording apparatus devised particularly for this type of work. There is no question but that beets of an average higher percentage of sucrose can be selected by their firmness ($r_{1\ 20} = +0.43 \pm 0.02$ and $r_{1\ 20\cdot 2} = +0.37$). It is easy to distinguish the excessively woody beets referred to by De Varies and Stoklasa by their extreme hardness. Under character 16 a similar set of partial correlation coefficients is described.

CHARACTER 21.—Various investigators have noted that the flat-crowned beets were of a higher percentage of sucrose. The height of the crown was measured in centimeters and represents the vertical distance from the first leaf scar to the apex of the crown. The relations of character 21 with characters 1, 2, and 3 are very significant. These coefficients (Tables 2 and 3) are similar to those for character 4 and should be similarly analyzed. (See character 4.)

CHARACTER 22.—In making estimates of the density of foliage (character 12) the estimate is always influenced by the size of the leaf as well as the number of leaves. Therefore, the density of foliage of beets with large leaves is overestimated, and this explains the negative relation between the density of foliage (character 12) and the percentage of sucrose. (See also correlation coefficient for character 6 and 1.) For this reason the writer devised the term "leaf density," which describes the ratio of the number of leaves on one parastichy (character 26) to the distance from the first leaf scar to the apex of the crown measured in centimeters. This ratio for the density of leaves on the sugar-beet crown corresponds exactly to the "ring density" of Seeliger (*42*). The leaf-density ratio describes the leaf density accurately. The leaf density is positively correlated with the percentage of sucrose, but negatively correlated with the weight and the sucrose content. This is consistent with the coefficients $r_{1\ 39}$, $r_{2\ 39}$, $r_{3\ 39}$ and with all other coefficients dealing with the number of leaves, the number of vascular-bundle rings, the diameter of crown, and the diameter of root. The leaf density increases with the percentage of sucrose, as is shown by the total and partial correlation coefficients for the entire material, all individual lines, and the means of individual

lines. The significant negative coefficients $r_{2\ 22\cdot1}$ and $r_{3\ 22\cdot1}$ indicate a strong association of characters 2 and 3 with character 22. In this particular instance the total coefficients $r_{1\ 22}$ and $r_{3\ 22}$ are more reliable than the partial coefficient $r_{1\ 22\cdot2}$ and $r_{3\ 22\cdot2}$ because the diameter of the root (which corresponds very closely to the weight) was considered in the original data (definition of leaf density), and the weight should not be held constant.

CHARACTER 23.—It was thought that beets having high percentages of sucrose had long leaves, though the results of Pritchard (*35*) indicate the reverse relation. The length of the leaf blade was judged by the length of the type leaf blade measured in centimeters. The coefficients of Tables 2 and 3 show that the length of the leaf blade was negatively correlated with the percentage of sucrose and positively with the weight and the sucrose content. The partial coefficients $r_{3\ 23\cdot1}$ and $r_{3\ 23\cdot2}$ show that sucrose content is significantly correlated with the length of leaf regardless of whether either character 1 or 2 was held constant.

CHARACTER 24.—Some investigators have noted a positive relation between the percentage of sucrose and the length of beet root, but others have noted no such relation. Florian (*8*) states that short beets have a higher percentage of sucrose. Briem (*4*) noted that the weight of the beet was directly related to the length of the root. The length of each root was expressed in centimeters. Table 2 shows that long beets have a slightly smaller percentage of sucrose but are heavier and have greater sucrose content. The peculiar character of the partial coefficients in Table 3 is due to the significant relations between the weight and the absolute length of root, and to the fact that holding the weight constant transforms the data to a relative basis. (See character 37.)

CHARACTER 25.—Plot (*34*) noted that beets of high percentages of sucrose had leaves with undulated margins, while Kneifel (*15*) and Pritchard (*35*) found no relation between the percentage of sucrose and the character of leaf margin. The margin of the leaf was described as to its undulated or flexuose character. The higher values were given for the more undulated margins. As the undulated characters of the margin vary with the age of the leaf, the type leaf was considered characteristic of each plant. It appears that beets having plain or straight-margined leaves have a slightly higher percentage of sucrose.

CHARACTER 26.—The number of leaves was found to be positively related to the percentage of sucrose by certain writers and negatively related by others. Sengbusch (*43*) found no difference in the number of leaves produced by the four brands of Klein Wanzlaben beets (ZZ, Z, N, and E), although they show marked differences as to the percentage of sucrose. Vychinski (*48*) states that beets of high percentages of sucrose have few leaves. The earlier investigators usually considered the number of leaves on the beet at harvest. As the first leaves fall off during the summer, the leaf count at any one particular time would not represent the total number of leaves produced by the plant. For this work the leaves on each beet were counted in June, August, and October, so that the numbers represent the total number of leaves produced during the entire vegetative period. The writer noted in 1923 that the leaves of beets (sugar

and feed types) were arranged along five prominent parastichies, which extend spirally from the first five leaf scars to the crown apex. Beets are of two types: (1) Those having the clockwise and (2) those having the counterclockwise parastichies. (Fig. 1.) It was found that the total number of leaves produced by any beet could be accurately and quickly determined by simply counting the average number of leaves and leaf scars per parastichy and multiplying by 5. This method checked very closely with the individual leaf counts made during June, August, and October. The correlation coefficients of Table 2 for character 26 with characters 1, 2, and 3 are significant. The partial coefficients of Table 3 indicate that the percentage of sucrose is not significantly correlated with the number of leaves, but

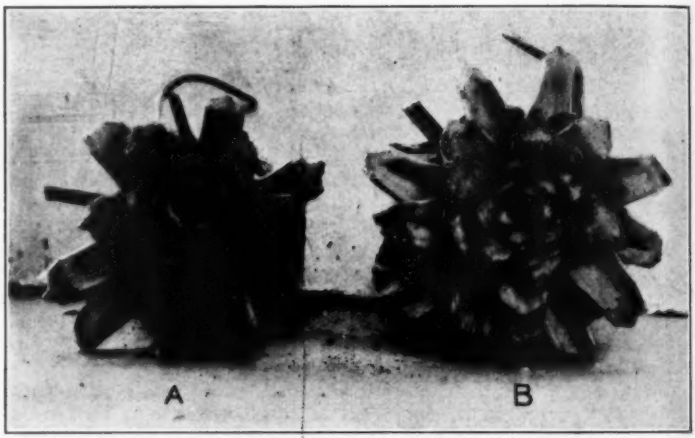

FIGURE 1.—A, Arrangement of leaves on a beet having clockwise parastichies and counterclockwise genetic spiral; B, arrangement of leaves on a beet having counterclockwise parastichies and clockwise genetic spiral

that the weight and the sucrose content are positively and significantly correlated with the number of leaves.

CHARACTER 27.—Certain investigators have noted a higher percentage of sucrose in beets having many vascular-bundle rings. Sengbusch (*43*) noted no difference between the four brands, ZZ, Z, N, and E as to number of rings. Pitsch (*32*) and Schneider (*40*) questioned the importance of ring numbers. The number of vascular bundle rings was in this instance counted with a hand lens from thin sections. The sections were cut from an intermediate point between the fleshy and suture surfaces and at the largest diameter of the beet body. The total correlation coefficients show no significant relation between characters 1 and 27. However, the partial correlation coefficient $r_{1\ 27.2} = +0.16 \pm 0.03$ shows that with beets of constant weight the percentage of sucrose increases slightly with the number of vascular bundle rings. This character is positively correlated with the percentage of sucrose, the weight, and the sucrose content. (See character 9.)

CHARACTER 28.—It is generally conceded that beets protruding high above the ground are low in percentage of sucrose. Vilmorin

(*46*) casts some doubt on this relation, and Saillard (*39*) noted that beets grew high out of the ground on certain types of soil with no resulting difference in the percentage of sucrose. Meyer (*19*) advocated the breeding of round protruding beets which carry less dirt and are easily dug even though the percentage of sucrose is slightly less. The position in the soil of each beet was measured in centimeters and represents the vertical distance from the soil level to the crown apex. The coefficients are similar to those for character 23. (See character 23.)

CHARACTER 29.—The purity coefficient is in general directly proportional to the percentage of sucrose. Urban (*44*) and Sengbusch (*43*) noted that some lines with lower percentages of sucrose actually had higher purity. The apparent purity was determined from refractometer reading and the percentage of sucrose. The coefficients for character 29 are very similar to those for character 16 and will admit of like analysis. (See character 16.)

CHARACTER 30.—The percentage of sucrose is positively correlated with the dry substance in the beet (see character 16) simply because the principal part of the dry substance is sucrose. The density of a sugar solution increases as its percentage of dry substance increases. Finally, the index of refraction is proportional to the density of the solution, and the refractometer reading is positively correlated with the percentage of sucrose. Many of the investigators cited under characters 11 and 16 contributed to the use of the refractometer for estimating the percentage of sucrose. The refractometer readings were either high or low in proportion to the percentage of sucrose, owing to the varying quantities of nonsugars peculiar to some beets. These points were strikingly brought out in the scatter diagrams. Table 2 gives the very significant value of $r_{1\ 30} = +0.81 \pm 0.01$, indicating that the percentage of sucrose increases with the refractometer reading. The regressions are linear, and the standard error of estimate ($Sy = 0.87$) is only 0.58, the value of the standard deviation. The total correlation coefficients describing this relationship between characters 1 and 30 for the separate lines were $+0.84 \pm 0.02$, $+0.86 \pm 0.02$, $+0.89 \pm 0.01$, $+0.86 \pm 0.02$, and $+0.85 \pm 0.02$. These results show in general that the percentage of sucrose increases directly with the refractometer reading. The correlation between characters 1 and 30 increases as the lines become more pure. If the purity of individual beets and the mean purity of lines are considered, this relationship becomes nearly perfect. The partial coefficients for this character admit of the same conclusions that were drawn for dry substance. (See character 16.)

CHARACTER 31.—The relative depth of the petiole groove represents the ratio of the depth to the width of the groove. The measurements were made from the petiole stamp. (See character 5.) The total correlation coefficients are not significant.

CHARACTER 32.—The relative flatness of the root was measured by dividing the fleshy surface (larger) diameter by the suture (smaller) diameter of the beet root. Character 32 is not significantly correlated with either character 1, 2, or 3.

CHARACTER 33.—The relative height of the foliage was represented by the proportion of the height of foliage to the diameter of foliage, measured in centimeters. The total correlation coefficients show that

the higher foliage types of beets have a slightly lower percentage of sucrose. (See character 19.)

CHARACTER 34.—Oblong leaves have been considered indicative of a high percentage of sucrose in the roots. The relative length of the leaf blade was defined as its length divided by its maximum width. The total correlation coefficients reveal no significant relations.

CHARACTER 35.—The relative length of leaf was expressed by the ratio of the blade plus petiole (length) to width of leaf. The coefficients in Table 2 for this character are not significant.

CHARACTER 36.—Vychinski (*48*) selected beets with long petioles, while Vivien (*47*) and Plot (*34*) favored the short robust petiole. Mass (*17*) considered this characteristic immaterial. The relative length of petiole was defined by the ratio of the length of petiole to length of leaf blade. Character 36 as here defined is not significantly correlated with either character 1, 2, or 3. Characters 33, 34, 35, and 36 were determined from measurements of the type leaf.

CHARACTER 37.—Certain investigators noted an increased percentage of sucrose in slender roots, while others found no significant relation. The relative length of root was expressed by the ratio of the length of the root to its maximum diameter. The weight of the beet has been found to increase with the length of the roots. (See character 24.) It will be noted that character 37 is positively correlated with character 1. (Compare with character 7.)

CHARACTER 38.—It is generally conceded that as the space between beets increases the percentage of sucrose decreases and the weight increases. Munerati, Mezzadroli, and Zapparoli (*22*) and Pellet (*31*) found no relation between the available space and the weight of the beet. Many writers find that the beet yield per unit area increases with the space up to about 40 by 30 cm. and then decreases. This expected condition is due to the factor of the number of beets entering into the results. This cultural problem will not be considered in this investigation, which deals primarily with individual beets. The amount of space available for each plant during its vegetative period in the field was referred to as relative space. These measurements were made for each plant and were expressed in square centimeters of area. The total correlation coefficients of Table 2 and for the individual lines show that the weight and the sucrose content of the beet increases with the relative space but that the percentage of sucrose changes negligibly. The partial correlation coefficients confirm these results.

CHARACTER 39.—The percentage of sucrose in the beet increases with its ring density. The ring density was expressed by the ratio, number of vascular-bundle rings (see character 27) to the radius of the beet root measured in centimeters (after Seeliger (*42*)). Table 2 shows that this character is positively correlated with the percentage of sucrose, but negatively with weight and with the sucrose content. The partial coefficients confirm these results except for coefficient $r_{1\ 39 \cdot 2}$ which has the fault of holding a character constant that has partly been considered in the original data. (See character 22.)

CHARACTER 40.—Mehais (*18*) and Munerati (*20*) report that rooty beets have a higher percentage of sucrose, while Saillard (*39*) and Vivien (*47*) noted that they had lower percentages of sucrose and that rootiness was due to soil conditions. Other investigators (*10*) found no relation between the percentage of sucrose and the rooty

character. The beets were classified according to the number of large forked roots and not in relation to the finer roots of the suture groove. There were very few rooty beets in the material investigated and the coefficients are questionable.

CHARACTER 41.—Sugar beets having rough skins are reported to have a higher percentage of sucrose (*47*). The term "roughness of skin," describes the general character of the skin and not the surface irregularities which are due to underlying tissue growth. Character 41 is positively correlated with each of the characters 1, 2, and 3. (See character 9.)

CHARACTER 42.—Some investigators have noted that beets having curly leaves are higher in sucrose, while others have found the reverse relation. Fruwirth and others (*10*) state that neither character 1 nor 2 is correlated with character 42. Rugosity was the term used to describe the curliness of the beet leaf surface. As the rugosity of the leaf surface depends upon the age of the leaf and the external conditions, the type leaf was considered characteristic of the plant, and the precautions outlined under characters 12 and 18 were observed. The total correlation coefficients for the entire material studied are given in Table 2. The total correlation coefficients expressing the same relations for the individual lines were very different. For instance, line 3 gave correlation coefficients $r_{142}=0.0$, $r_{242}=0.0$, and $r_{342}=0.0$, showing that characters 1, 2, and 3, were absolutely independent of character 42. This was expected, because every beet in line 3 was uniformly alike for character 42.

CHARACTER 43.—The leaves of sugar beets vary as to the degree of neutral gray shade that appears along with their green color. (See character 9.) Table 2 indicates that beets having leaves of darker shade have higher percentages of sucrose. Character 43 is positively correlated with each of characters 1, 2, and 3. (See Tables 2 and 3 and character 9.)

CHARACTER 44.—Sugar marks are indicative of high percentages of sucrose. Sugar marks seem to be characteristic of sugar beets and are very rarely noticed on feed beets. These marks consist of corrugations on the beet surface that extend horizontally away from the root sutures. Character 44 is also positively correlated with each of the other characters 1, 2, and 3. (See Tables 2 and 3 and character 9.)

CHARACTER 45.—Mass (*17*) and Pritchard (*35*) noted that thick-leafed beets had higher percentages of sucrose than thin-leafed beets, whereas Bolotoff (*2*), Knauer and Doerstling (*14*), and Schribaux (*41*) noted the reverse. The thickness of the leaf was measured with a micrometer reading to 0.001 of an inch. The measurements were always taken from a particular locality on the type leaf because the thickness of sugar-beet leaves varies with the age of the leaf and changing external conditions. The partial coefficients indicate no significant relationships.

CHARACTER 46.—The majority of investigators have noted an increased percentage of sucrose in beets with spirally turned sutures. In these investigations no significant differences were noted as to characters 1, 2, or 3 between beets with straight and those with turned sutures.

CHARACTER 47.—It is generally acknowledged that the foliage weight per beet is negatively correlated with the percentage of sucrose but positively correlated with the weight of beet and the sucro

content. Schribaux (*41*) pointed out that the weight of leaves has increased with the breeding of beets having higher percentages of sucrose. The weight of the foliage represents only the quantity of foliage that was on each beet at the time of harvest and in no way represents the total weight of foliage produced by the beet during its vegetative period. Owing to the fact that the weight of the foliage on a beet depends upon its maturity, the weather conditions, and the losses of leaves during cultivation, as well as upon the total foliage produced, these results should be considered as to maturity of beet in connection with any physiological relation between characters. Thus the negative relation between characters 1 and 47 may either indicate that beets have low percentages of sucrose because they have retained most of their leaves and are not mature, or the reverse, that beets have high percentages of sucrose because they are mature and have lost most of their leaves. After due allowance is made for these two contingencies, there remains the possibility that beets having high percentages of sucrose may produce relatively less foliage.

CHARACTER 48.—Schneider (*40*) reported higher percentages of sucrose in beets having wide leaves, but Pritchard (*35*) reported the reverse relation. The width of the type leaf was considered characteristic of the plant. The total correlation coefficients of Table 2 show that the percentage of sucrose decreases as the width of the leaf increases. There is no doubt that the percentage of sucrose is negatively correlated with the width of leaf, because of the strong positive relation between characters 2 and 48 and the negative relation between characters 1 and 2. Further, if characters 1 and 48 are considered and the weight of the beets held constant, there appears to be no significant relation between the width of leaf and the percentage of sucrose ($r_{1\ 48\cdot 2} = -0.08 \pm 0.03$, Table 3.) This conclusion was significantly reached by a slightly different method. (See character 34 and Table 2.) The partial correlation coefficients should be considered in the light of the statements under character 15.

CHARACTER 49.—Pritchard (*35*) noted that beets having wide petiole grooves had smaller percentages of sucrose. The width of the petiole groove was measured at the point of minimum cross-section area of the petiole. (See character 5.) The total correlation coefficients are given in Table 2. The partial correlation coefficient $r_{1\ 49\cdot 2}$ supports Pritchard's conclusion.

CHARACTER 50.—Most breeders have associated the deep suture groove of sugar beets with high percentages of sucrose. The width of the suture was estimated by comparison with its depth. The coefficients for character 50 in Table 2 are not significant.

INTERPRETATION OF RESULTS

The multiple correlation coefficient of the sucrose content with the percentage of sucrose and the weight has the significant value of 0.965. This indicates that three-fourths of the variability has been considered, and the standard error ($S_{3\cdot 12} = 19.28$) is only about 25 per cent of the standard deviation for the sucrose content. As the sucrose content depends upon the percentage of sucrose and the weight of the beet root, the partial correlation coefficients for the percentage of sucrose with each of the selection characters listed in column 1 of Table 3 when constant for weight are given in column 2,

and the partial coefficients for weight with each of these characters when constant for percentage of sucrose are given in column 3 of the table.

Any permanent increase in the percentage of sucrose in the beet is of fundamental importance because a high percentage of sucrose is of value in itself, and the writer has shown under character 2, that the sucrose content increases with the percentage of sucrose ($r_{13 \cdot 2} = +0.52 \pm 0.02$). The partial correlation coefficients $r_{1\ 11 \cdot 2}$, $r_{1\ 16 \cdot 2}$, $r_{1\ 17 \cdot 2}$, $r_{1\ 24 \cdot 2}$, $r_{1\ 29 \cdot 2}$, $r_{1\ 41 \cdot 2}$, and $r_{1\ 43 \cdot 2}$ indicate that the percentage of sucrose of beets may be improved by selecting beets of increased density, dry substance, shoulder extension, hardness of tissue, length of root, purity, refractometer reading, roughness of skin, and darkness of leaf color. The negative coefficients $r_{1\ 21 \cdot 2}$ and $r_{15 \cdot 2}$ indicate that these beets should have flat crowns and petioles of small cross-section area.

In the consideration of lines of beets that are relatively uniform for percentage of sucrose, the partial correlation of the weight with the sucrose content constant for percentage of sucrose ($r_{23 \cdot 1} = +0.96$) indicates a very significant relationship, because the selection of beets with characters significantly correlated with the weight will quite invariably be significantly correlated with the sucrose content. (Compare coefficients of columns 3 and 4, Table 3.) In this connection the selection characters 15, 18, 21, 24, 26, and 28, should be found of service. Some of these characters will present no difficulty because they are not significantly correlated with the percentage of sucrose. Others (character 18, for example) are positively correlated with the weight ($+0.54$) but negatively (-0.15) with the percentage of sucrose. This indicates that if the weight was increased by selecting beets of high foliage area the percentage of sucrose might be reduced. The alternative would be to increase the foliage area (character 18) by selecting beets with a large number of leaves (character 26) rather than beets with few large leaves (character 6).

In the study of lines that are uniform for either character 1 or 2, it is important to note the correlation of the sucrose content with each of the selection characters when either character 1 or 2 is held constant. The partial correlation coefficients for sucrose content with each of the selection characters constant for either percentage of sucrose (column 4) or weight (column 5) are given in Table 3.

The selection characters 9, 27, 41, 43, and 44 are positively correlated with the percentage of sucrose, the weight, and the sucrose content regardless of whether character 1 or 2 is held constant The indications are that these particular characters should be further investigated to determine whether these relationships are or are not due to true genetic differences.

DISCUSSION

The correlations presented here are generally considered to be of a physiological nature and may be of value for indirect selection. Indirect selection permits of an easy determination of one character in place of the difficult determination of a valuable character if these two characters show a large significant correlation. Of the many selection characters studied, only a few were found sufficiently associated with the valuable characters of the beet to be of immediate

breeding value. As all selection characters were adapted to field conditions these few selection characters are of unusual consequence to the character and cost of sugar-beet breeding. The data presented show that the more important selection characters are of value for indirect selection and may be used to supplement the polariscope.

A review of the statistics on the percentage of sucrose and the weight of sugar beets shows that the coefficient of variation for weight is about five times greater than that for percentage of sucrose. These variations are largely of a modificational nature, being due to environmental conditions. Tables 2 and 3 show that weight is positively correlated with relative space (character 38) but percentage of sucrose is not significantly correlated with relative space. In the calculation of the partial correlation coefficients constant for weight, the larger part of these modifications is eliminated, and the consideration of these partial correlation coefficients as given in Table 3 should be utilizable for the selection and improvement of sugar beets.

As the weight and the sucrose content of the beet root increase with the space available per beet in the field, all beets planted for selection work should be spaced uniformly at the optimum distance. Indications are that this optimum distance varies with different strains of sugar beets. A uniform spacing of breeding beets at once obviates the necessity for discarding beets adjoining blank spaces in the row and the laborious determination and recording of the available space allotted each beet in the field.

As sugar beets tend to become uniform for one character or another and to fall into classes of high percentage of sucrose or of high weight, partial correlation may be applied directly to sugar-beet breeding. For example, sugar-beet strains that are of uniform weight or of high weight should be bred for an increased percentage of sucrose. The aim is to maintain the desirable weight characteristic of the strain and at the same time to increase the percentage of sucrose. Possibilities are offered by the method of partial correlation which provides a means of holding certain characters constant while other varying characters are considered. For instance, the partial correlations of the percentage of sucrose with each of the selection characters constant for weight (column 2, Table 3) indicate which selection characters should be used to improve the percentage of sucrose in this particular strain while its weight characteristic is retained. On the other hand, sugar-beet strains that have high percentages of sucrose and poor weight should be studied as to the partial correlation coefficients listed in column 3, Table 3. These partial correlation coefficients indicate which selection characters should be used for the improvement of sugar-beet strains that may be either of poor quality or weight. The choice of selection characters to be studied will depend upon the interrelation of the characters involved, as was illustrated on page 540.

As a strain becomes more uniform for a particular character, this character decreases in value as a correlation character for selection. When the strain is homogeneous for this character its correlation with other characters will show no correlation. (See character 42.) However, this particular character then becomes very useful as an identification mark of its strain.

SUMMARY

The material studied was statistically complete as noted. The combined material (496 individual beets) consisted of five previously known lines grown and examined under optimum conditions, measured in standard units, and analyzed immediately after harvest. All the data were recorded for each individual beet and the statistics calculated for the individual lines as well as the entire material. Scatter diagrams were made, and more than 500 total, multiple, and partial correlation coefficients were calculated, considering the percentage of sucrose, the weight, and the sucrose content of the beet with 47 selection characters.

A reliable and rapid method for estimating the number of sugar-beet leaves is given.

The selection character leaf density is defined as an exact method for stating the density of leaves on the sugar-beet crown.

The effects of the unequal spacing of beets in the field were eliminated by statistical methods.

In general the total correlation coefficients for each separate line are similar to the corresponding total correlation coefficient for the combined material.

Sucrose content was considered in relation to all of the selection characters because it is one of the most important characteristics of the sugar beet.

It appears that the physiological correlation of the percentage of sucrose with the weight of the beet is negative and significant. This conclusion is strengthened by the fact that nearly all the characters which are positively correlated with the percentage of sucrose are negatively correlated with the weight of the beet. Even though it was possible to show that the sucrose content increased with the percentage of sucrose when the weight of the beet is held constant, no partial correlation was calculated that showed a positive relation between the percentage of sucrose and the weight. In this connection it is obvious that if a line of beets is uniform for either weight or percentage of sucrose there would be no correlation between these characters. (See discussion, p. 541.)

Percentage of sucrose was found to be significantly and positively correlated with sucrose content.

As many of the correlations indicate a strong association of weight with sucrose content, the weight of the beet should be given more consideration.

These data showed that the percentage of sucrose is not significantly correlated with the available space given a beet in the field. As the weight and sucrose content of the beet increase with the available space, sugar beets planted for selection work should be spaced uniformly to the optimum distance for the strain.

As the multiple correlation coefficient and unpublished data indicate that the percentage of sucrose and the weight of beets are very important characteristics for determining the value of sugar beets, the partial correlation coefficients for each of these characters with each of the more important selection characters are given in Table 3. The partial correlation coefficients for sucrose content with these selection characters are also given.

The calculation of the partial correlations presented in this paper made it possible to state the net association of sugar-beet characteristics. These coefficients were used to analyze the selection characters which were highly correlated among themselves and to eliminate the effect of uncontrolled conditions. It also was found possible to apply partial correlation directly to sugar-beet breeding, because the percentage of sucrose and the weight of sugar beets seldom increase simultaneously.

The partial correlation coefficients of Table 3 also indicate which of the selection characters should be considered for the improvement of special strains of sugar beets that may be of poor weight or quality. They show likewise, that, aside from the polariscope, the refractometer reading (30) is the most reliable selection character to consider in improving the percentage of sucrose in the beet. Along with this, characters 17, 20, 24, 29, 41, and 43 should be considered.

The coefficients show that the number of leaves is the most important selection character influencing the weight of beets. Characters 13, 15, 18, 22, 24, 28, 39, and 48 might also be considered.

Characters 9, 27, 41, 43, and 44 were found to be positively correlated with each of the characters 1, 2, and 3.

The selection characters are adapted to field studies, and they can be utilized as a valuable aid in the improvement of sugar beets.

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A MOSAIC OF WHEAT TRANSMISSIBLE TO ALL CEREAL SPECIES IN THE TRIBE HORDEAE[1]

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INTRODUCTION

The green and the yellow types of mosaic discussed in this paper occur on winter wheat and winter rye in several counties in Illinois and Indiana (*3, 4*)[2] and on winter wheat in Davidson County, N. C. An extreme dwarfing or rosette condition is associated with the green mosaic on several varieties of winter wheat, and on this account the malady has previously been designated by the name rosette disease. Although this name characterizes the rosette condition, it appears that the more inclusive name mosaic is more appropriate.

The green and the yellow types of mottling associated with this malady may be caused by one virus or by distinct viruses. This point will be discussed later. Throughout this paper they are treated as a single disease on account of their close association in the infested soils that have been studied.

This mosaic shows certain characteristics which differ from those manifested by the tropical mosaic occurring on sugarcane, Indian corn, and other species belonging largely to the higher tribes of the grasses as classified by Hitchcock (*1*).

The most notable difference is in the matter of soil transmission. This mosaic of the small grains is perpetuated from year to year by means of virus present in the soil stratum (*3, 4, 10, 11*). As yet experiments have not supplied the evidence that might lead to the determination of the exact relationship between the soil and the virus. It is possible that subterranean vectors such as nematodes, soil-borne insects, other animal forms, or even fungi, may carry the virus. It is possible also that the virus is held by the finely divided particles of soil and organic matter.

The occurrence of this mosaic depends largely on the fall and winter conditions that influence growth and dormancy. The disease does not develop when susceptible varieties are sown in infested soil during the summer, and it is difficult to obtain infection experimentally from expressed juice when the inoculated plants are propagated in warm locations.

This mosaic has not been transmitted experimentally by *Aphis maidis* or by aphids which commonly feed on wheat. Its host range as determined thus far is within the tribe Hordeae, which is one of the lower tribes of grasses.

[1] Received for publication Sept. 24, 1929; issued March, 1930. These studies were conducted in part in cooperation with the Wisconsin Agricultural Experiment Station during the 7 years the writer was stationed there.

[2] Reference is made by number (italic) to "Literature cited," p. 556.

METHODS

Infested black-silt soil was obtained near Granite City, Ill., and infested brown clay-loam soil was obtained near Wanatah, Ind. All tests were made in these virus-infested soils, which were first transported to Madison, Wis., and later to the Arlington Experiment Farm, Rosslyn, Va. These soils have maintained a high degree of infestation. Some lots of the black-silt soils have been used each year for 10 years. In 1929 some of these showed signs of reduced infestation.

In previous experiments it was found that susceptible strains of Harvest Queen wheat and other varieties do not usually develop mosaic when sown in mosaic-infested soil in the spring when temperatures are rising; accordingly all sowings, including the spring types, were made in the fall at the period most suitable for sowing the fall grains.

In some instances each variety or species of host was tested in a row 4 feet long, and in others the rows were 2 feet in length.

All spring types were carefully mulched with straw or hay to prevent or reduce winterkilling. Although this mulching assisted greatly, some varieties were winterkilled entirely or in part.

Several Egyptian wheats and a barley were tested in 1929. These were obtained from the Egyptian Ministry of Agriculture through L. E. Melchers. Tests on these were of interest, as certain varieties found susceptible to rosette mosaic in the United States were resistant to a similar disease which occurs in the lower Nile region, thus indicating that the Egyptian disease may be due to another causal agent.

RESULTS

Tables 1 and 2 give results of all the susceptibility tests made with cereals other than winter common wheat and rye. The results of control tests made in virus-free soil are not tabulated. In all but one case the plants grown in the virus-free soil were free from mosaic and rosette. In one test on Red Winter spelt the control plot was close to virus-infested soil and 1 plant out of 46 developed yellow mosaic.

From Table 1 it is seen that all of the species and varieties of the different species of Triticum which were tested developed mosaic to some degree.

Three varieties of winter barley—Nakano Wase, Wisconsin Winter (C. I.[3] 2167), and Han River—developed mosaic. The last two varieties showed a very small amount of the disease in 1926, but none developed in subsequent tests. Nakano Wase was the most susceptible barley tested. Persian 1, a spring barley, developed mosaic in 1929. No rosette occurred in barley.

All the varieties of winter oats tested were free of mosaic. They are: Bicknell (C. I. 206–155), Black (C. I. 691), Culberson (C. I. 273), Custis (C. I. 2041), Lee (C. I. 2042), Winter Turf (C. I. 431), Winter Turf (C. I. 453–4), Fulghum (C. I. 708), Fulghum (C. I. 699–2015), and Red Rustproof (C. I. 1079).

Both green and yellow types of mottling occurred on the small grains grown in virus-infested soil. The green type (fig. 1, B and C) was the most common. The yellow streaks, stripes, and blotches (fig. 1, D, E, G, H) were prevalent in Red Winter spelt and in certain selections of Currell, Illini Chief, Mediterranean, and Goens common wheats.

[3] C. I. indicates a serial accession number of the Office of Cereal Crops and Diseases.

TABLE 1.—*Species and varieties of the genus Triticum, other than winter varieties of T. vulgare, which have been tested and found susceptible to a mosaic of winter wheat in experiments conducted at the Arlington Experiment Farm, Rosslyn, Va., in 1927, 1928, and 1929*

Species and variety	Growth habit	C. I. No. or source	Percentage of mosaic infection in—		
			1927	1928	1929
T. vulgare:					
Federation	Spring				75
Haynes Bluestem	do				6
Hard Federation	do				10
Hindi D	do	Egypt.			100
Marquis	do	3641	6	4	100
Sinai 1	do	Egypt.			5
Sonora	do	3036			100
T. compactum:					
Coppei	do	3088		4	10
Hybrid 60	do	5024		4	10
Hybrid 128	Winter	4512		75	80
T. turgidum:					
Titanic	do	5535		90	
Winter Alaska	do	5873		90	
T. durum:					
Acme	Spring	5284			50
Beladi 26	do	Egypt.			100
Marouani	do				100
Mindum	do	5296			25
Palestine 12	do	Egypt.			100
Pentad	do				50
T. dicoccum:					
Black Winter	Winter	2337	2	12	1
T. spelta:					
Alstroum	do	1773		2	5
Bearded Winter	do	1724	8	6	60
Red Winter	do		100	98	100
T. polonicum:					
Abyssinian	Spring		3		100
Unnamed	do			60	95
T. monococcum:					
Unnamed	Winter		99	90	100

TABLE 2.—*Reaction of varieties of barley (Hordeum sativum) tested for susceptibility to a mosaic of winter wheat in experiments conducted at Madison, Wis., in 1926 and at the Arlington Experiment Farm, Rosslyn, Va., in 1927, 1928, and 1929*

Habit and variety	C. I. No. or source	Percentage of mosaic infection in—			
		1926	1927	1928	1929
Spring habit:					
Persian 1	Egypt.				40
Winter habit:					
Alaska	4106	0	0	0	
Han River	2163	4	0	0	0
Nakano Wase	754	4	4	16	95
Orel	351	0	0	0	
Pidor	901	0	0	0	0
Tennessee Beardless No. 6	2746	0	0	0	
Tennessee Winter	257	0	0		
Tennessee Winter Selection 66	3546	0	0		
Wisconsin Winter	2167	4	0	0	0
Do	2159		0	0	

The mottled areas of yellow mosaic consist of short or long, narrow or wide, patches which tend to parallel the veins of the leaf or the leaf sheath. Long streaks or stripes sometimes occur. In some cases these streaks consist of a series of short streaks that have become united. (Fig. 1, D, E, H.) In other cases continuous wide yellow bands appear between the veins of the leaf, leaving the veins and a narrow margin of the lamina tinged with light green, as shown in Figure 1, F.

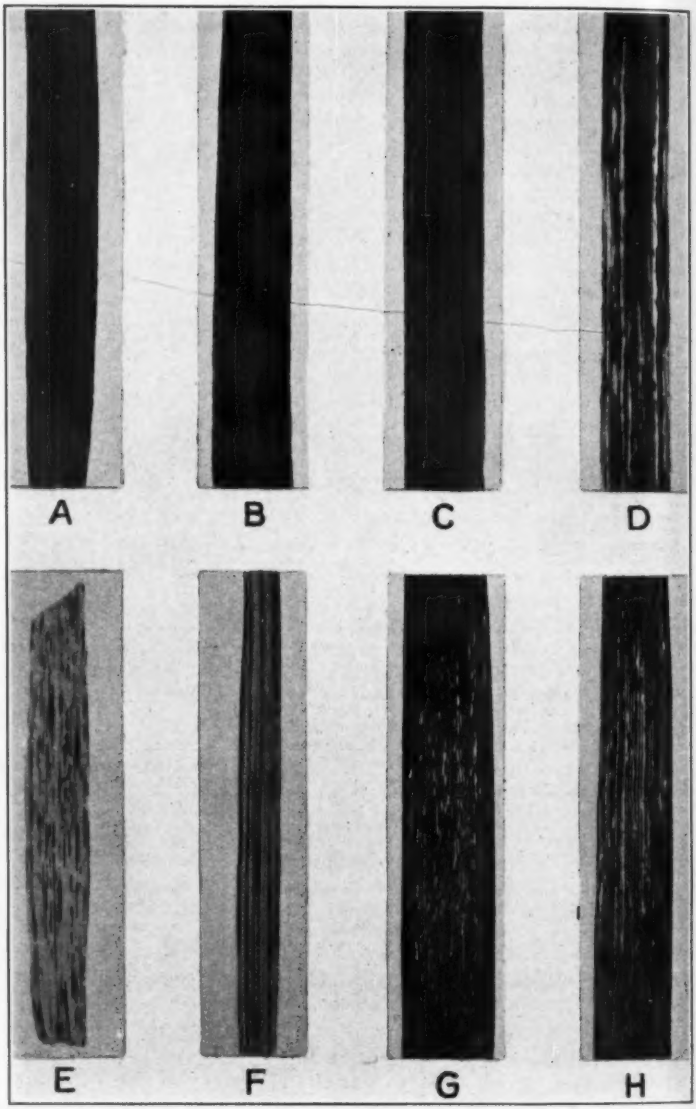

FIGURE 1.—Mosaic on small grains: A, Mosaic-free leaf of winter wheat; B, green mosaic on winter rye, showing a predominance of light-green surface; C, green mosaic on Harvest Queen winter wheat, showing a predominance of dark-green surface; D and E, yellow mosaic on Currell winter wheat; F, yellow mosaic on Polish wheat; G and H, yellow mosaic on a selection of Currell. A, C–H, × 1; B, × 2

The yellow patches, shown in Figure 1, D and E, frequently lose all of the carotin and xanthophyll pigments and become white or brown.

The yellow banding illustrated in Figure 1, F, has been observed only on Polish wheat. However, a less severe form (fig. 2, C) has been observed on individual plants of several varieties of common wheat grown in virus-infested soil.

The mosaic-free Polish wheat plants grown in the virus-free soil showed a considerable amount of light-green banding or striping on the leaves, as shown in Figure 2, B. This condition is more or less prevalent among certain varieties of mosaic-free common wheat, and individual plants have been observed which showed an unusual tendency to produce these bands under favorable environmental conditions. The extent to which this tendency may predispose plants to develop the yellow-stripe mosaic illustrated in Figure 1 is being studied.

Plants affected by severe yellow mosaic (fig. 1, D, E, and F) become stunted, but no excessive proliferation has been observed in these cases. In Currell and some strains of Illini Chief winter common wheat, and in Red Winter spelt, yellow mosaic frequently prevents the maturing of the seed. The grain from these plants is shriveled and of little or no value to the grower. In most cases the germination power of such seed is so low that it is necessary to conduct all selection studies in noninfested as well as in infested soil to insure obtaining seed for further experimentation.

In these experiments cell inclusions were abundant in the mottled leaves of all species and varieties of small grains affected by green mosaic, but they were usually very difficult to find or not present in tissues showing signs of yellow mosaic.

Modified or intermediate types of rosette have been observed in individual plants of rye and common wheat, as illustrated in Figure 3, B. These intermediate forms have also been observed in certain selections of Harvest Queen, Illini Chief, Mediterranean, and Goens common wheats. Affected plants become stunted at various times after typical rosette has made its appearance. The plants become dark green, thus masking most of the mosaic. If conditions are favorable such plants may send out secondary shoots, but they have been observed very rarely. These symptoms may be governed by factors that regulate infection and by genetic factors.

Selection studies show that many of the disease-free plants of the several species tested represent resistant types. Continual selection of yellow types and green types of mosaic shows that strains can be developed which produce a high percentage of plants susceptible to the mosaic type being selected.

DISCUSSION

Green and yellow types of mosaic have been observed on wheat outside of the infested areas given in this paper. In 1923 G. L. Peltier showed the writer a green mosaic on wheat growing in the experimental plots at Lincoln, Nebr., which was indistinguishable in general appearance from the green mosaic occurring in Illinois and Indiana. No rosette was associated with this mosaic, but this may have been due in part to the variety of wheat.

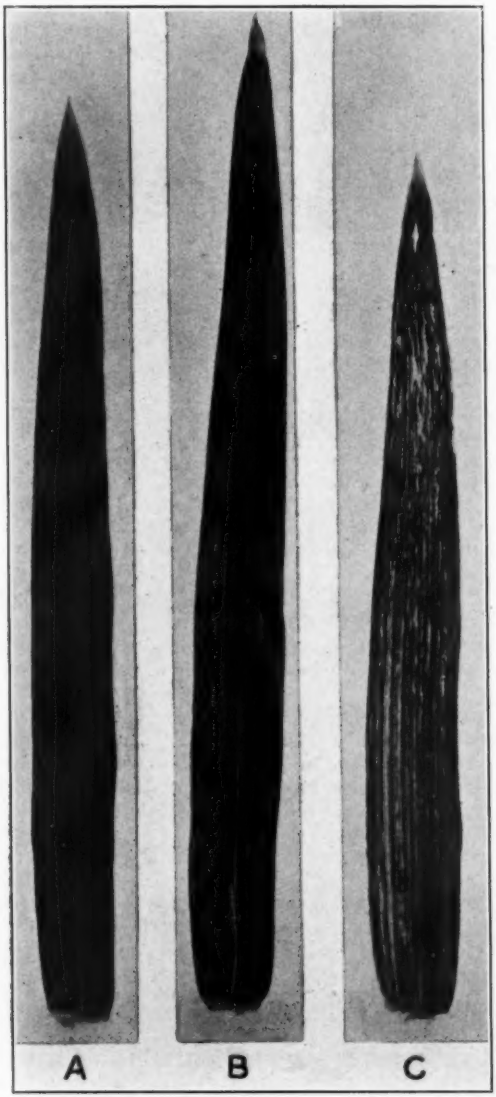

FIGURE 2.—A, Mosaic-free leaf of winter wheat; B, light-green banding associated with certain varieties and selections of Triticum when not infected with the virus of mosaic; C, yellow mosaic combined with the banding illustrated in B. All × 1

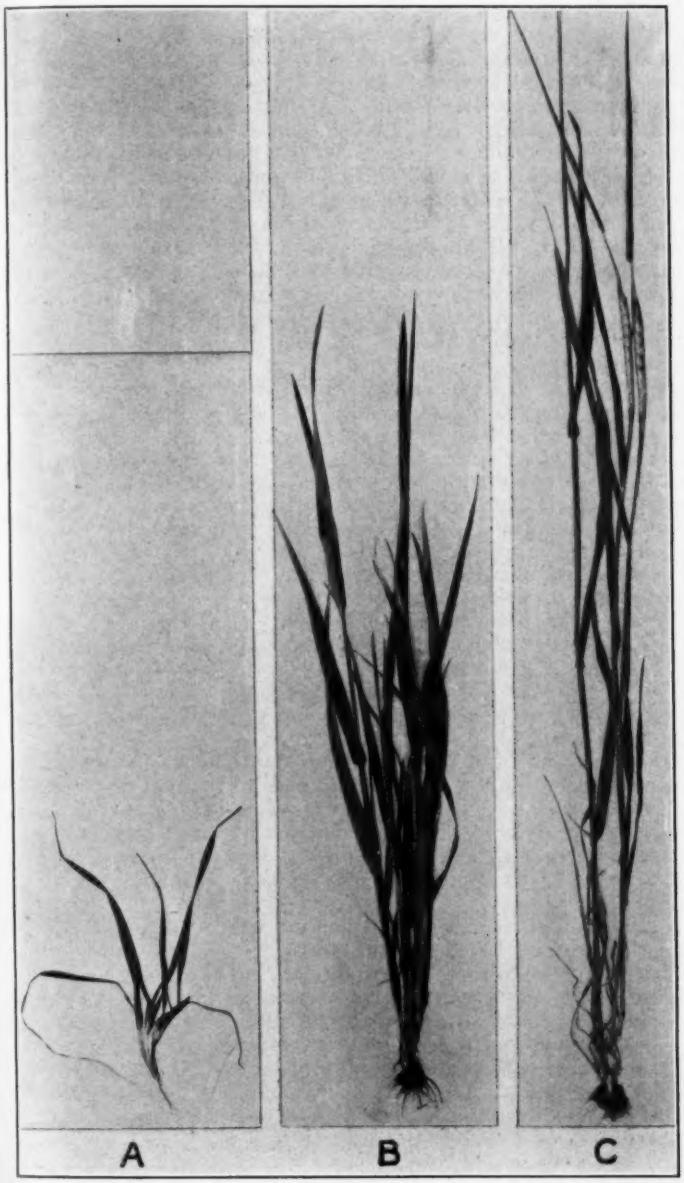

FIGURE 3.—Harvest Queen wheat. A, Typical rosette dwarfing, which appears early in the spring. The healthy control plants were three-fifths taller than the rosetted plants. B, Dwarfing and proliferation, which appear late in the spring or early in the summer. C, Healthy control plant collected with B. All × ¼

Peltier reports that he transmitted this mosaic to wheat and to Indian corn by means of infectious juice and to one corn plant by means of an unidentified aphid.[4] It seems evident, therefore, that the disease is infectious.

The writer made microscopic examinations of the mottled tissues of wheat plants collected at Lincoln, Nebr., but was unable to locate the typical cell inclusions which have always been found in similar tissues of plants with mosaic in Illinois and Indiana. These observations have led the writer to consider that the green mosaic in Nebraska is different from that occurring on wheat farther east.

In 1927 H. B. Humphrey collected a green mosaic on wheat near Rockville, Md. Mottled leaf tissues contained a large number of cell inclusions, but they were not exactly like those associated with the green mosaic which is the subject of the present investigation. The significance of this difference between cell inclusions must await further study.

In the spring of 1929 the writer's attention was called to a yellow mottling and stuntedness in winter wheat growing in the wheat propagation nursery of the Kansas Agricultural Experiment Station at Manhattan. Within a few days of this observation a similar mottling was observed in winter wheat on the agronomy farm of the Nebraska Agricultural Experiment Station located near Lincoln. No typical green mosaic was found associated with these yellow disorders.

The general appearance of these disorders was indistinguishable from the yellow mosaic that occurs in Illinois and Indiana. However, it is possible that these diseases are not due to a common cause.

Some of the patterns of yellow mosaic on wheat are strikingly similar in appearance to the streak and stripe diseases of Indian corn described and illustrated by Storey (*9*) and Stahl (*8*), respectively.

The mosaic situation in the small grains is suggestive of that occurring among several varieties of potatoes (*7*) and other species of Solonaceae (*2*, *5*, *6*).

Several forms of foliage mottling and dwarfing types of virus diseases which occur on the potato represent distinct diseases caused by distinct viruses. The work on the mosaics of tobacco, tomato, and *Nicotiana glauca* (*2*, *6*) also shows clearly that there are several distinct viruses which produce different types of mosaic symptoms, and also that yellow mosaics are commonly associated with some of the green types (*5*, *6*).

It is possible that several distinct viruses cause mosaic and other abnormalities in wheat, and that the cereal species, varieties, and selections vary as to their susceptibility to these different viruses. It is possible also that certain green and yellow types of wheat mosaic are commonly associated, as has been found to be the case with some of the green and yellow mosaics of tobacco (*5*, *6*).

These are interesting analogies, but there are points concerning the grass mosaic under discussion which make it appear that they can not be pressed too far until methods of experimentation have been improved.

The interpretation of these varied expressions of mosaic, rosetting, and dwarfing depends to a large extent on the genetic purity of the

[4] HASKELL, R. J., and WOOD, J. I. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1922. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 27, p. 164–266, illus. 1923. [Mimeographed.]

host plants that produce the different types of symptoms. Long-continued selection for the botanical and agronomic characteristics of wheat varieties seems not to guarantee homozygocity for the factors determining resistance or susceptibility to mosaic and rosette. It has been found necessary to develop new lines pure for the disease characteristics that are under study. It also is essential that the small amount of natural crossing which occurs in wheat be eliminated by carefully bagging the heads of the plants.

Methods for separating and purifying the viruses of the grass mosaics must be developed.

SUMMARY

Infection experiments have been carried out with small grains sown in virus-infested soils transported from areas in Illinois and Indiana where wheat mosaic occurs.

The disease has been produced in the following species, all of which are of the tribe Hordeae: *Triticum vulgare*, *T. compactum*, *T. turgidum*, *T. durum*, *T. dicoccum*, *T. spelta*, *T. polonicum*, *T. monococcum*, *Hordeum sativum*, and *Secale cereale*.

All susceptible species tested gave evidence indicating that resistant strains were present.

Mosaic developed appreciably in cereals with the spring-growth habit only when they were sown in the fall at the time the fall types of cereals were sown. Careful mulching of the spring types reduced winterkilling.

Typical mosaic rosette occurred only among a limited number of varieties of winter common wheat. Strains of common wheat which develop 100 per cent typical mosaic rosette have been selected.

Green and also yellow types of mottling and yellow streaking or striping occur on plants growing in the infested soils studied.

By continual selection it has been possible to produce several lines of small grains which produce a very high percentage of yellow mosaic, and others which produce a high percentage of the green type.

The yellow types of mosaic cause the plants to become dwarfed late in the spring and the seed to be shriveled and practically valueless.

All varieties and species of small grains which produce mosaic when grown on the virus-infested soils under study were examined for cell inclusions. In all cases of green mosaic examined typical cell inclusions were abundant in the mottled leaves. Mottled leaves with yellow mosaic showed very few cell inclusions, and frequently none could be found.

A green mosaic with which no cell inclusions have been identified occurs in Nebraska.

A green mosaic occurs on wheat in Maryland with which cell inclusions occur, but these show characteristics somewhat different from those of the cell inclusions associated with the green mosaic occurring in Illinois and Indiana.

A yellow mottling occurs, apparently independent of green mosaic, on wheat at Manhattan, Kans., and at Lincoln, Nebr. The external signs of this malady are indistinguishable from those manifested by the yellow mosaic under study. No cell inclusions have been identified in the tissues of specimens obtained at Manhattan and Lincoln.

It is not possible to interpret all of these observations satisfactorily at this time. Several distinct viruses may cause mosaic and other disorders in the small grains. Some of the yellow and green mosaics of wheat may be commonly associated with each other, as has been found with some of the mosaics of the Solonaceae. Genetic factors within the plant may influence the symptoms produced by a given virus.

Rosette has never been observed on plants independently of mosaic, and all evidence indicates that rosette is a phase of the mosaic disease.

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REPETITIONAL DIPLANETISM IN THE GENUS PHYTOPHTHORA[1]

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INTRODUCTION AND HISTORY

In a recent abstract (*10*)[2] concerning a prominently papillate species of Phytophthora different from *P. erythroseptica* Pethyb., as the cause of at least occasional instances of pink rot of potato (*Solanum tuberosum* L.) tubers in the United States, reference was made to the frequent manifestation of diplanetism by the fungus. Secondary motile zoospores were set forth as being delivered directly through an evacuation tube, or liberated from a 1-spored sporangium produced terminally on a delicate germ sporangiophore. Mention was made of the rather striking reproductive arrangement often resulting when zoospores imprisoned within an ordinary sporangium exhibit the latter type of development, as well as of the prevalence of diplanetism in congeneric forms, an appropriate instance being cited in another species which had been isolated from diseased potato tubers in the United States, and which was similarly capable of causing pink rot.

In spite of considerable study devoted to species of Phytophthora during more than a half century, the production of a second swimming spore from a previous one, without the interposition of a vegetative phase, appears, as far as the writer is aware, to have been recorded only once—in Sawada's recent account (*22*) of his *Phytophthora melongenae*. To be sure, according to Gäumaun (*11, p. 80*), Murphy observed

> in *Phytophthora infestans* the shedding of the membrane, as in Dictyuchus; the zoospores which have found no suitable substrate come to rest and surround themselves with a membrane. After a certain time they again slip out with the same reniform appearance and swarm further.

From the publication (*16, p. 459*) cited, it is not apparent that the text permits such interpretation, as Murphy seemingly is not describing either any condition suggestive of Dictyuchus or emergence of a secondary swimming spore from an encysted stage. His account of the production of secondary conidia on germ tubes of zoospores is interesting nevertheless in relation to the phenomenon of repetitional diplanetism:

> When the zoospores germinate under favorable conditions, the germ tube is of such enormously greater capacity than the original spore, and continues increasing in length (even though not completely filled with protoplasm), or at least remains alive and vigorous-looking so long, that a saprophytic existence is at once suggested. * * * They, too, have the faculty of producing "secondary" conidia, an observation which does not seem to have been recorded previously. * * * Subsequently in a preparation originally set up under sterile conditions and kept for five days at 10°–15° C., in which there had been abundant zoospore formation, the almost universal production of "secondary" conidia by zoospores situated near the edge of the cover glass was observed. * * * These new conidia

[1] Received for publication Nov. 13, 1929; issued March, 1930.
[2] Reference is made by number (italic) to "Literature cited," p. 572.

were borne on the ends of long spirally twisted tubes. They contained all the protoplasm, even of the branches, where such were present. In form they exactly resembled those produced by conidia being asymmetrical and provided with prominent papillae and yellow oil-drops, but they were much smaller, although having a considerably greater volume than the original zoospore.

The increased volume of the secondary conidium implies, apparently as an essential feature of the kind of development discussed, an expansion of material that could scarcely be attributed to purely reproductive processes.

A presumably similar course of development was noted later by Godfrey (*12*) in a study of one of the rhubarb (*Rheum rhaponticum* L.) foot-rot parasites described by him as *Phytophthora parasitica* var. *rhei:*

> Within an hour or two after motility ceases zoospores often begin to germinate. * * * Germination in water sometimes results in the formation of small conidia, normal in appearance, as shown in F. These may produce zoospores, or may germinate by germ tubes and continue the vegetative growth.

The small conidium illustrated in the Figure F referred to is represented by a structure that would seem to exceed in volume the zoospore from which it originated by no less than eight times. The widening of the more distal portion of filament suggests too that the product of the zoospore may have attained such extension that it might perhaps have been more appropriately designated as a young mycelium rather than as a germ tube.

The reproductive development of the zoospores of *Phytophthora melongenae* described by Sawada does not appear to have been seriously disturbed through the intervention of vegetative growth. As the original account is in Japanese, a quotation from a somewhat free translation of the text [3] may be appropriate:

> After swimming about for 30 minutes or an hour the zoospore loses its cilia, comes to rest, and rounds up as a spherical body, 10 to 11μ in diameter. On resting approximately 30 minutes it germinates by a single delicate germ tube, though in one instance two tubes were found produced. The germ tube either develops extensively, or it ceases to elongate and gives rise to a terminal conidium. The latter measures usually 11 to 12 by 8 to 9μ, though examples measuring as much as 13 to 16 by 10 to 13μ occur. Probably the former contains a single zoospore while the latter contains two. These small conidia open at the apex to liberate zoospores.
>
> The zoospore which fails to make its way out of the conidium comes to rest inside and germinates, the germ tube sprouting through the papilla which already is open, or penetrating the conidial wall. In case the tube penetrates the wall, it frequently soon ceases elongation to produce a terminal conidium which germinates by liberating a zoospore.

Among the illustrations accompanying Sawada's text are three figures showing zoospores that have germinated by delicate germ sporangiophores each bearing terminally a minute, obviously 1-spored sporangium. In one of these figures (*22, pl. 3, fig. 3*) the structures are lying free; in another (*22, pl. 3, fig. 6*) the zoospores, four in number, imprisoned within the primary sporangial membrane, have each thrust their germ sporangiophore through the papillary opening to produce a minute sporangium externally; and in the third (*22, pl. 3, fig. 7*) four zoospores out of a larger number of imprisoned encysted structures have again each produced a minute zoosporangium internally, the germ sporangiophore here, however, in all instances perforating the wall of the primary sporangium.

[3] The translation was prepared by Sabura Katsura.

To another figure (*22, pl. 3, fig. 4*) showing two evacuated zoospore membranes, each with an ample papillary opening, is attached the explanatory legend: "Resting zoospore producing a zoospore directly."

TREATMENT FAVORING DIPLANETIC DEVELOPMENT

As repetitional diplanetism seems to be of some importance in the biology of various species of Phytophthora, the present discussion supplementing Sawada's observations may not be excessive. The conditions favoring the production by encysted zoospores of a second swimming stage without the interposition of a vegetative phase apparently are the same as those that favor zoospore formation generally. In order that the necessary development might take place on a scale large enough to permit ready observation, it was found advantageous, after inducing the production and discharge of a generous supply of ordinary sporangia, to maintain conditions suitable for continued discharge. This could not usually be accomplished by mounting aerial sporangiferous material from artificial cultures on a microscope slide and covering with a cover glass, or by placing the material in a sealed hanging-drop preparation, or by cultivating in a Van Tieghem cell.

With the forms discussed in the present paper more satisfactory results were obtained through direct use of growth in Lima-bean agar, though for other types, like *Phytophthora cryptogea* Pethyb. and Laff., and *P. cinnamomi* Rands, this substratum was obviously less suitable. Portions of plate culture containing actively growing mycelium were removed to sterile Petri dishes, cut into pieces of convenient area, and irrigated with sterile distilled water in such a way that the upper surface of the agar was moistened without being inundated. As a result of such procedure, the papillate potato tuber parasite, for example, gave rise in the course of 12 to 15 hours to an extraordinarily heavy crop of sporangia, these being comparable in uniformity in size and shape with the sporangia produced in nature by *P. infestans* (Mont.) De Bary or *P. phaseoli* Thaxter. As long as the cover of the Petri dish was kept in place zoospore production occurred only in negligible quantity. On the removal of the cover the familiar changes recorded by numerous observers—cleavage of protoplasm, writhing movement, progressive individualization, swelling of the papillary substance—began and proceeded simultaneously in practically all the fully grown sporangia, with the result that after about a half hour very rapid liberation of zoospores lasting about 10 or 15 minutes ensued everywhere.

As an access of fresh air seemed to be almost the only consequence of the removal of the cover, Murphy's observation on the importance of oxygen in zoospore production in *Phytophthora infestans* was thus accorded confirmation in the behavior of the papillate pink-rot parasite. A similar response to ventilation was shown in preparations of most of the other congeneric organisms included in the present study. Material kept under observation and hence subjected to illumination from a microscope lamp gave rise to the swimming stage with noticeably greater rapidity than similar material in the same container but not exposed to special illumination.

SEQUENCE OF EVENTS IN DIPLANETIC DEVELOPMENT

With an abundance of zoospores available at the beginning, and additional quantities supplied by the dehiscence of succeeding crops of sporangia, the development leading to a secondary swimming stage could be looked for several hours later, suitable conditions being maintained meanwhile by admitting, if need be, fresh air from time to time, and by cautious replacement of water lost by evaporation. An early stage in such development of the papillate pink-rot organism is represented in Figure 1, A, *h* and *i*, the zoospores, after rounding up and becoming encysted as spherical bodies 8 to 12μ in diameter, having produced a dome-shaped protrusion measuring mostly 3 to 4.5μ in basal diameter and generally approximately the same in length. Until the protrusion attains its definitive size, it is filled, like the structure from which it originates, with protoplasm containing fine granules in rather open arrangement.

Since in considering the direct delivery of secondary motile zoospores the various forms included in the present study in which such delivery has been demonstrated exhibit no departure from a same uniform sequence of events, the series of stages (fig. 1, K, *l–n*) drawn from material of the rhubarb foot-rot fungus, *Phytophthora parasitica* var. *rhei* Godfrey, will illustrate the pertinent developmental stages revealed in the papillate pink-rot parasite as well as in other congeneric types. The first internal change readily noticeable consists in the retraction of the granular contents from the protuberance so that the latter appears to be filled only with clear liquid. (Fig. 1, E, *l*.) Soon thereafter, if not at the same time, the protoplast can be

EXPLANATORY LEGEND FOR FIGURE 1

A.—Unnamed form with papillate sporangia isolated from decaying potato tubers, possibly referable to *P. parasitica:* *a*, Extensive development of miniature sporangia from zoospores imprisoned within the second of a series of three sporangia; *b–e*, miniature sporangia produced from free encysted zoospores; *f*, an empty miniature sporangium; *h*, *i*, encysted zoospores with evacuation tubes; *j–s*, empty cyst envelopes after escape of secondary swimming spores.

B.—A nonpapillate species isolated from diseased Idaho potato tubers, possibly to be referred to *P. erythroseptica:* *a–f*, Encysted zoospores, each showing a vacuole and an evacuation tube; *g*, motile zoospore at moment of liberation from cyst envelope; *h*, *i*, evacuated cyst envelopes.

C.—*P. melongenae:* *a*, Large sporangium containing an evacuated cyst envelope; *b*, sporangium containing imprisoned zoospores, of which all except one have given rise to germ sporangia; *c–j*, germ sporangia produced by free encysted zoospores; *k*, free germ sporangium after dehiscence; *l*, encysted zoospore with evacuation tube; *m*, *n*, evacuated cyst envelopes.

D.—*P. parasitica* derived from diseased cotton boll: *a*, Extensive development of miniature sporangia from imprisoned zoospores; *b*, sporangium containing two empty cyst membranes, one manifesting direct dehiscence, the other the production of a germ sporangium; *c*, sporangium with two evacuated cyst envelopes, and a secondary motile spore again to be retained within parent sporangium; *d*, sporangium with extensive development of germ sporangia from imprisoned zoospores; *e*, *f*, successive stages in development of germ sporangium; *g*, germ sporangium borne on an unusually long sporangiophore; *h*, *i*, germ sporangia after dehiscence; *j–n*, encysted zoospores with evacuation tubes; *o*, encysted zoospore immediately before liberation of secondary spore; *p*, escape of secondary zoospore from cyst envelope; *q–z*, *a′*, *g′*, evacuated cyst envelopes.

E.—*P. parasitica* var. *rhei:* *a*, Sporangium containing two evacuated cyst envelopes; *b*, sporangium showing extensive production of miniature sporangia by imprisoned zoospores; *c*, sporangium with four imprisoned zoospores, three having produced germ sporangia, the other having discharged a secondary motile spore directly; *d–j*, germ sporangia after dehiscence; *k–n*, successive stages in development of secondary zoospore directly within cyst wall; *o*, zoospore with unusually long evacuation tube; *p*, another zoospore in same state as *l*, immediately preceding evacuation; *q–x*, evacuated cyst envelopes.

F.—Unnamed form isolated from diseased Honeydew melon: *a*, Germ sporangium produced by encysted zoospore; *b*, evacuated germ sporangium; *c–n*, evacuated cyst envelopes.

G.—Unnamed form isolated from diseased sugar-beet root in Utah: *a–c*, Encysted zoospores with broad protuberances of dehiscence; *d–m*, evacuated encysted envelopes.

H.—Unnamed form isolated from diseased tomato fruit in California: *a–e*, Encysted zoospores with developing evacuation tubes; *f–o*, evacuated cyst envelopes.

I.—*P. fagi:* *a*, Secondary motile spore after emergence being held fast by cilium adhering to inner surface of cyst wall; *b–e*, evacuated cyst envelopes.

J.—*P. citrophthora:* *a–c*, Germ sporangia produced by free encysted zoospores; *d*, *e*, evacuated germ sporangia; *f*, encysted zoospore with well-developed evacuation tube; *g*, *h*, evacuated cyst envelopes

K.—*P. hibernalis:* *a–f*, Germ sporangia produced by encysted zoospores; *g–p*, evacuated germ sporangia.

L.—*P. cactorum:* *a*, Profuse development of germ sporangia by encysted zoospores imprisoned in a large sporangium; *b–d*, germ sporangia produced by free zoospores; *e*, germ sporangium after evacuation; *f*, encysted zoospore with evacuation tube; *g–k*, evacuated cyst envelopes.

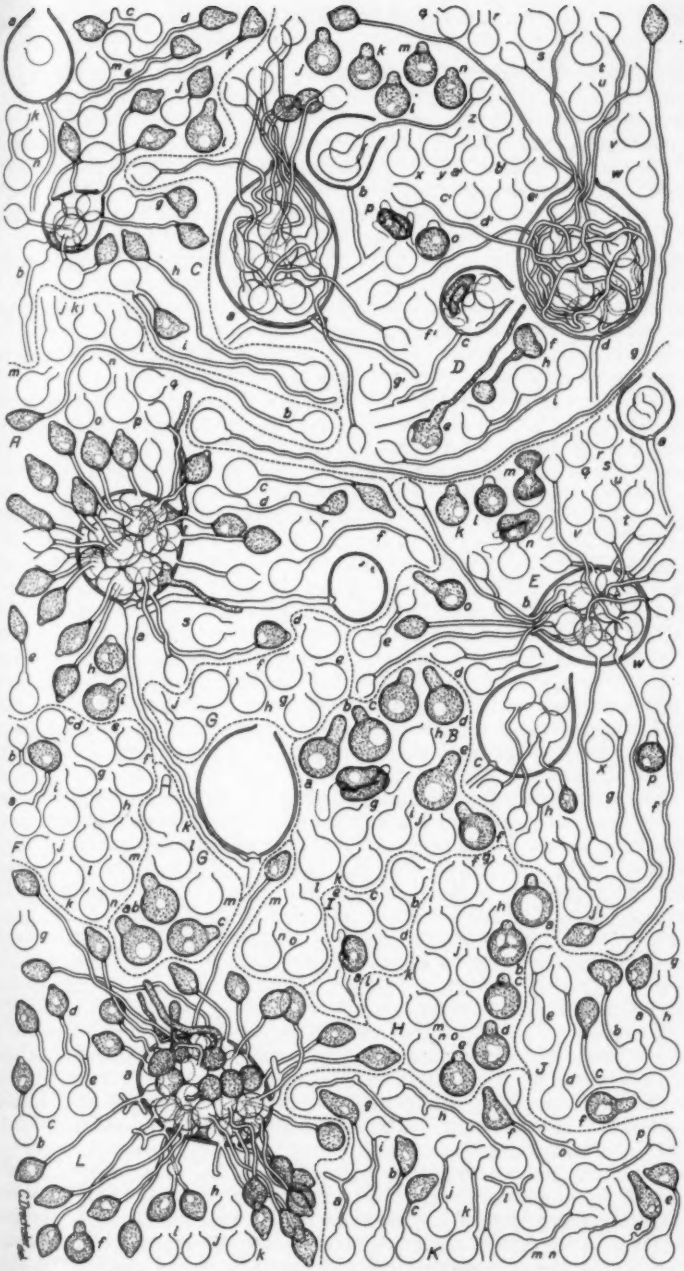

FIGURE 1.—Repetitional diplanetism in various species of Phytophthora. All parts drawn with the aid of the camera lucida. × 500
(For explanatory legend see opposite page)

observed to have drawn away slightly here and there from the cyst membrane. Writhing movements similar to those that are associated with sporogenesis in the large sporangia distinctive of the genus, now become apparent. Some time after movement becomes perceptible, usually not exceeding 10 or 15 minutes, the tip of the protrusion gives way and the protoplasmic contents pass out through the isthmus (fig. 1, E, *m*), becoming integrated at the orifice as a biciliate zoospore of the same type as that from which it was derived (fig. 1, E, *n*). The passage of the protoplast through the relatively wide neck is ordinarily accomplished in 3 or 4 seconds, while the pause between its delivery and its swimming away as a motile zoospore occupies about 1 second as a rule, rarely more than 2 seconds.

As the zoospore produced is not apparently distinguishable from those coming directly from an ordinary large sporangium, only the empty cyst membrane with its papillary orifice or open evacuation tube—a structure much more familiar to students of the Saprolegniaceae than to investigators dealing with the general run of fungi pathogenic to economic plants—is left behind as visible evidence of the developmental phenomenon described, which for convenience might be referred to as "direct" diplanetism. The evacuation tube, of course, represents the membrane surrounding the cylindrical portion of the protuberance of dehiscence, and is generally relatively short, often measuring about 1μ in length. (Fig. 1, A, *n*, *q*, *r*.) Greater lengths, however, are not rare (fig. 1, A, *k*, *l*, *m*, *o*, *p*, *s*), although a condition like that shown at A, *j*, the most extreme encountered in any of the forms studied, in which the length of the tube equals or perhaps slightly exceeds the diameter of the spherical part, must be regarded as decidedly unusual. Obviously the empty cyst envelopes remaining as evidence of the occurrence of direct diplanetism correspond to the two structures shown in Sawada's Plate 3, Figure 4, to which reference has already been made.

The other kind of repetitional diplanetic development illustrated by the Japanese author and described in his text somewhat more fully, was, as has been stated earlier, also abundantly represented in material of the papillate pink-rot fungus. The encysted primary zoospore served as origin of a filament from 1 to 1.3μ in diameter growing out either immediately from its periphery (fig. 1, A, *c*) or more frequently from the tip of a much stouter protuberance (fig. 1, A, *b*, *d*, *e*) similar to the modification involved in the direct dehiscence already described. After attaining a length ordinarily varying between 5 and 25μ, though sometimes exceeding 50μ, the delicate filament ceased to elongate and swelled terminally into an expansion which increased in size with the migration into it of granular materials. Eventually the protoplasmic contents were completely transferred to the terminal structure, which then invariably became delimited from the filament by a septum. The distinctive shape, often obpyriform, and the terminal papillate modification that soon became apparent at once characterized the structure externally as a miniature sporangium. This characterization was consistently borne out when, after perceptible retraction of the contents from the confining membrane, especially in the apical region, and writhing movements of the usual duration, the apical beak yielded, and the protoplast squeezed through the orifice and swam away from the empty sporangium (fig. 1, A, *f*) as a biciliate zoospore. The latter was not distinguishable from

zoospores of primary origin, although in pronounced instances it seemed to be marked by perceptible inferiority in size. The developmental feature incident to the production of a secondary motile zoospore formed singly within a miniature sporangium borne on a delicate germ sporangiophore arising directly from an encysted zoospore or from an ineffective protuberance of dehiscence may conveniently be termed "indirect" diplanetism.

As has been suggested, indirect diplanetism is often revealed conspicuously in the case of zoospores that have failed to escape from the large primary sporangia usual in species of Phytophthora. Incomplete discharge of the latter structures, to which reference is made by numerous investigators, is observable in some measure in almost every lot of material of any member of the genus. While the reasons for frustrated dehiscence are not always obvious, in the writer's experience instances became especially numerous when the upper surface of the irrigated Lima-bean agar over which the sporangia were distributed either became too dry or was flooded to excess. It seems probable, therefore, that deficiency in moisture on the one hand and deficiency in oxygen on the other are frequently causes. In any case the imprisoned zoospores, after moving about within the sporangium for a considerable period with such vigor as their number and the limited space permitted, finally came to rest and rounded up inside.

According to observations of numerous students, further development proceeds by the production of vegetative germ tubes that perforate the sporangial wall and thus gain access to the exterior. Such vegetative development is common, especially when the structures concerned remain submerged rather deeply. Under more favorable circumstances, as has been mentioned, imprisoned zoospores of the papillate pink-rot fungus were found to give rise individually to single germ sporangiophores that perforated the sporangial wall, ordinarily without evident diminution in diameter, or passed through the papillary orifice. A terminal miniature sporangium that subsequently liberated a single secondary swimming spore was produced in each case. In instances where the imprisoned zoospores numbered a score or more, or where only a relatively small proportion had been discharged normally, the encysted bodies were packed so closely that their membranes partly adhered to one another, and the germ sporangiophores were thrust forth in bristling array. (Fig. 1, A, *a*.) The resulting arrangement provided a reproductive apparatus of distinctive appearance.

Direct diplanetism, while usually not as abundantly manifested in the case of imprisoned zoospores of the papillate pink-rot fungus as indirect, nevertheless is observed here in considerable measure. As the circumstances attending such occurrence are evidently similar in all species where it has been demonstrated, figures drawn from congeneric forms may be taken as adequately illustrative. When only one (fig. 1, C, *a*) or a few (fig. 1, E, *a*) zoospores are imprisoned, so that the cysts come to lie within the sporangial envelope under conditions not greatly different, with respect to crowding, from those encountered outside, all of the secondary swimming bodies may be directly discharged by way of an evacuation tube and gain access to the exterior of the sporangial wall by passing through the papillary opening. Direct repetitional development may occur even in cases where the papillary opening happens to be effectually blocked by one

of the cysts, as for example, in the instance represented in Figure 1, D, C. The obstructing cyst readily discharged its protoplast by the happily oriented evacuation tube directly through the papillary orifice, while the secondary zoospore from the less fortunately placed cyst was prevented from egress and as a result after protracted effort rounded up inside. In most instances, perhaps, the majority of the zoospores imprisoned within a sporangium give rise to a secondary swimming stage by the indirect method of development, while one or a few, especially of those most favorably situated with reference to crowding and accessibility to the papillary opening, manifest direct dehiscence. (Fig. 1, D, *b*, and E, *c*.)

PARTICULARS OF DIPLANETISM IN VARIOUS SPECIES OF PHYTOPHTHORA

Diplanetism is not restricted to a few members of the genus Phytophthora. Its occurrence in a fungus isolated from diseased potato tubers originating in Idaho in 1922 was recorded earlier. Mainly because of the identity of the host material from which it was derived, its ready pathogenicity to potato tubers on inoculation into wounds, and the absence of any papillate modification in the ovoid sporangia, the fungus was tentatively referred to *P. erythroseptica*. The American nonpapillate pink-rot fungus was never found to produce sexual structures in any substratum on which it was tried out, though parallel cultures of the organism received from the Centraalbureau voor Schimmelcultures at Baarn, Netherlands, under the label "*P. erythroseptica*" (presumably authentic, inasmuch as it was contributed originally by G. H. Pethybridge), often yielded an abundance of normal oospores. The sporangia of the American form exceeded those of the European parasite, especially in length, measurements of 200 of these bodies developed on irrigated Lima-bean agar giving an average length of 53.5μ and an average diameter of 30.2μ as compared with 44.5μ and 29.7μ, respectively, calculated for the organism obtained from abroad. A rather strong proliferous tendency manifested in the American fungus by the sporangiophore growing through the base of one sporangium after its evacuation, to produce another farther on, often yielded a series of three or four empty sporangial envelopes borne on the same axial sporangiophore. The zoospores of the fungus are relatively large, measuring in the subspherical encysted form about 12μ in diameter. Development leading to a second swimming stage (fig. 1, B, *g*) is begun by the production of a papilla of dehiscence usually 4 to 6.5μ in diameter and 2 to 8μ in length (fig. 1, B, *a–f*), the empty envelope being provided with a rather wide evacuation tube of variable length (fig. 1, B, *h–o*). Indirect diplanetism through the development of a miniature 1-spored germ sporangium has never been observed in the fungus under consideration.

Irrigated pieces of Lima-bean agar culture of the parasite isolated by Ocfemia (*17*) from diseased eggplant (*Solanum melongena* L.) fruits in the Philippines, and treated by him as being identical with *Phytophthora melongenae*, provided an abundant display of diplanetism. (Fig. 1, C, *a–n*.) Production of miniature sporangia (fig. 1, C, *c–j*) appeared much more frequently than direct liberation of the secondary motile bodies, both when the encysted primary zoospores were free and when they were imprisoned within the large sporangia.

(Fig. 1, C, *a*, *b*.) The secondary miniature sporangia produced by two of the seven imprisoned zoospores shown in Figure 1, C, *b*, did not give rise to a swimming stage directly, but gave rise instead to tertiary sporangia. Additional irregularities were found in occasional branching of the germ sporangiophore, the short diverticulum not set off by a cross wall and the relatively long element eventually delimited by a septum, shown in Figure 1, *C*, *c* and *i*, respectively, providing representative examples.

Abundant diplanetism was evident in irrigated material of a fungus communicated by S. F. Ashby as a strain of *Phytophthora parasitica* Dastur derived from cotton bolls in Monserrat. It, presumably, was the same organism further referred to in a publication (*1*) as having been isolated by E. M. Wakefield. (Fig. 1, D, *a–e'*.) Many of the larger sporangia of the fungus, suggesting chlamydospores in their almost subspherical shape, are provided with a relatively thick envelope. Apparently because of its substantial character, the latter structure is not readily perforated by the germ sporangiophores of imprisoned zoospores, which consequently are constrained to wind about extensively before emerging either by eventually piercing the wall or more often by passing through the papillary opening. (Fig. 1, D, *a*, *d*.) Failure to reach the exterior is rare, since the germ sporangiophores, even when not confined, often attain an unusual length. (Fig. 1, D, *g*.) The broad basal protuberance from which many (fig. 1, D, *e*, *f*, *g*, *i*), though not all (fig. 1, D, *h*), of the germ sporangiophores arise give an impression as if direct development of a second swimming stage is first attempted, followed in case of failure by the more indirect development. That the acquisition of cilia within the membrane of the encysted structure (fig. 1, D, *o*) and the escape of the biciliate zoospore (fig. 1, D, *p*) are not rare is attested by the presence of numerous empty cyst envelopes with evacuation tubes of variable length, abundantly scattered about free in appropriately treated material, as well as within empty primary sporangia (fig. 1, D, *b*, *c*).

Close similarity to the cotton-boll fungus in structures associated with diplanetism as well as in cultural characteristics and morphology of the sporangium was revealed in eight cultures that had been isolated from the tomato, five having been obtained from fruits affected with buckeye rot, two from stems affected with decay near the soil line, and the other from a rootlet showing decay at the tip. Such similarity is in harmony with the assignment of the buckeye-rot parasite, originally described from the tomato as *Phytophthora terrestris* Sherb., to *P. parasitica*. About the same similarity was evident also in a fungus derived from rhubarb affected with foot rot in the District of Columbia in September, 1928, and presumably to be identified with the form described by Godfrey as *P. parasitica* var. *rhei*. Here, also, frustrated dehiscence of the zoospores was provided for through the appearance of a secondary swimming stage, by direct discharge of the motile body (fig. 1, E, *a*), by production of a germ sporangium (fig. 1, E, *b*), or by both types of development (fig. 1, E, *c*). The sporangial wall usually was not as thick as the more strongly indurated homologous structures of the cotton-boll parasite, its perforation by the germ sporangiophores entailing only relatively little coiling of the filamentous structures. Otherwise production of the germ sporangia (fig. 1, E, *d–j*) took place much as in the cotton-boll

fungus, and the direct liberation of zoospores from the encysted bodies (fig. 1, E, *k–n*, *p*), following a sequence of stages already described, yielded evacuated cyst envelopes in large numbers (fig. 1, E, *q–x*).

A moderate display of diplanetism was observed in irrigated Lima-bean agar preparations of the species of Phytophthora, the isolation of which from a honeydew melon (*Cucumis melo* var. *inodorus* Naud.) fruit affected with decay was reported in a brief abstract (*9*). Production of germ sporangia (fig. 1, F, *a*, *b*) occurred less frequently than liberation of the secondary motile spores from the primary encysted ones (fig. 1, F, *c–n*). When the latter were somewhat irregular in shape, often as a result of rounding up under crowded conditions in insufficient water, their capacity to give rise to a second swimming stage was not noticeably impaired. (Fig. 1, F, *d*, *f*.)

A species of Phytophthora isolated by C. M. Tompkins from a mature sugar beet (*Beta vulgaris* L.) root originating at Price, Utah, in 1927, and greatly resembling the form isolated from affected Idaho potato tubers already discussed with respect to cultural characters and to morphological features pertaining to the sporangium, resembled the latter fungus also with respect to repetition of the swimming stage. The relatively large encysted primary zoospore, measuring usually about 12μ in diameter, put forth a wide papillary protuberance (fig. 1, G, *a–c*) that functioned in permitting the direct liberation of a secondary motile spore, leaving the empty cyst envelope (fig. 1, G, *d–m*) provided with a usually short evacuation tube. Germ sporangia arising from encysted zoospores were never observed. The funnel-shaped evacuation tube shown in G, *j*, and the short cylindrical diverticulum with the distal part delimited by a septum shown in G, *k*, represent departures from more regular development. In the absence of a more adequate description of the beet parasite, measurements of 50 sporangia produced on irrigated Lima-bean agar gave a range in length of 34 to 75μ, with an average of 58.3μ, and a range in width of 27 to 40μ, with an average of 33.1μ. After the evacuation of the somewhat narrow ovoid zoosporangium by means of an apical modification not protruding from the general contour as a recognizable papilla, the sporangiophore often continued growth through the empty envelope to produce another sporangium farther on. Repetition of such renewal of growth frequently resulted in three or four empty envelopes being found in series on a single axial supporting filament.

A species of Phytophthora isolated by G. B. Ramsey in October, 1928, from California tomato (*Lycopersicum esculentum* Mill.) fruits with brownish or brownish-purple lesions on the distal region showed great similarity in its manifestation of diplanetism to the congeneric fungi from Utah sugar beets and Idaho potatoes. The primary zoospore, measuring usually from 10 to 12μ in diameter after encystment, produced a protuberance of ample size (fig. 1, H, *a–e*) by means of which the motile secondary spore was directly liberated, leaving behind the empty cyst envelope with open evacuation tube (fig. 1, H, *f–o*). The fungus with its ovoid nonpapillate sporangia having an average length of 47.3μ and an average diameter of 30.9μ—these values being calculated from 50 measurements, using irrigated Lima-bean agar—was apparently specifically distinct from any hitherto reported on the tomato. The absence of protruding papillae from the sporangia readily sets it apart from the types assignable to

Phytophthora parasitica (*P. terrestris* and the stem-girdling fungus reported by Reddick (*20*)), as well as from *P. infestans* (Mont.) De Bary and *P. mexicana* Hotson and Hartge (*13*). Compared with an authentic culture of *P. cryptogea* Pethyb. and Laff. received from the Imperial Bureau of Mycology, the California fungus showed a considerably faster rate of growth, a noticeably smaller diameter of mycelial threads, and a vastly greater production of sporangia. Its close affinity and possible identity with the parasites from sugar beets and from Idaho potatoes were indicated by similarity in beheavior and appearance when the three organisms were given similar treatment in parallel cultures and preparations.

Repetitional diplanetism in moderate measure was revealed in irrigated Lima-bean agar preparations of a fungus received in artificial culture from the Centraalbureau voor Schimmelcultures as *Phytophthora fagi* Hartig. The secondary zoospore in all cases observed was formed directly within the cyst envelope. In a number of instances the protoplast, after its passage through the papilla of dehiscence, was held tethered near the orifice for several minutes by one of the cilia adhering to the inner surface of the empty cyst membrane apparently with sufficient firmness to resist the pull exerted by the other cilium. (Fig. 1, I, *a*.) Since *P. fagi* has frequently been regarded as probably identical with *P. cactorum* (Cohn and Leb.) Schroet., and indeed resembles it closely in shape and size of sporangium, it may be of interest to note that in the fungus under consideration a secondary swimming stage was not observed to arise indirectly through the production of a miniature germ sporangium—a type of development exceedingly frequent in *P. cactorum*. The encysted zoospores of the fungus from the Centraalbureau and the empty envelopes left behind after their evacuation (fig. 1, I, *b–f*) appeared generally slightly larger than the corresponding structures of the related organism, the average diameter being about 10μ in the former, as compared with 9μ in the latter. Differences in cultural features were also evident, the fungus received from the Netherlands growing less than one-fourth as rapidly in linear extension as the other when cultivated, for example, on Lima-bean agar, and its thallus revealing a densely branching habit on that medium as compared with an openly disposed one. Any trustworthy opinion regarding the relationship of *P. fagi* and *P. cactorum* should, however, be based on a study of a dozen or more cultures of the beech (*Fagus sylvatica* L.) seedling parasite, preferably from sources not too close together geographically.

Several strains of *Phytophothora citrophthora* (Sm. and Sm.) Leon., all derived presumably from California lemon (*Citrus limonia* Osbeck) fruits affected with brown rot, exhibited repetitional diplanetism in ample measure in irrigated Lima-bean agar preparations. The secondary motile stage was developed both indirectly by the production of germ sporangia (fig. 1, J, *a–e*) and directly by the acquisition of cilia within the cyst membrane. The evacuation tube associated with the latter process was usually of moderate proportions. (Fig. 1, J, *f–h*.) As in certain other species, the germ sporangiophore frequently was present as a delicate prolongation of a broader basal protuberance, the latter evidently being an unsuccessful evacuation tube. (Fig. 1, J, *a*, *c–e*.)

Diplanetism was abundantly manifested in irrigated Lima-bean agar preparations of a fungus received from the American Type Culture

Collection as *Phytophthora hibernalis* Carne, and representing, presumably, an authentic type of the organism described recently (*3*) as the cause of brown rot of citrus in Australia. The secondary swimming stage was brought about through the production in extraordinary numbers of miniature germ sporangia (fig. 1, K, *a–f*) and their subsequent evacuation (fig. 1, K, *g–p*). No instances of development of motility directly within the cyst envelope were observed, though the frequent origin of the delicate germ sporangiophores from a broader basal protuberance (fig. 1, K, *a–c, f, m, n, p*) suggested the possibility that such development may have been attempted but came to naught, owing perhaps to unsuitable conditions. The production of a tertiary germ sporangium from the secondary one shown in Figure 1, K, *a*, and a certain tendency toward branching evident in some sporangiophores (fig. 1, K, *a, d, g, h, l, o*) constitute irregularities.

Reference has been made to the frequent exhibition of diplanetism in irrigated preparations of *Phytophthora cactorum* through the production of miniature sporangia. The important part played by such development in the liberation of swimming zoospores in instances of frustrated dehiscence of the large sporangia is illustrated in Figure 1, L, *a*, showing 28 germ sporangia borne on pedicels thrust through the confining sporangial wall from a corresponding number of imprisoned cysts. Similar development ensues in quantity also with primary zoospores that have encysted unconfined in their surroundings after a normal period of active swimming. (Fig. 1, L, *b–e*.) The secondary zoosporic stage may arise, too, through the fashioning of the motile body within the cyst membrane, as shown by the presence of a papillate protuberance in many encysted structures (fig. 1, L, *f*), later persisting on the evacuated envelope as a cylindrical modification of varying length (fig. 1, L, *g–k*). While all strains of *P. cactorum* show close similarity in diplanetic development, it may not be amiss to record that the illustration shown in Figure 1, L, was made from a Lima-bean agar preparation of one of the several strains from diseased stems of *Lilium pyrenaicum* Gouan, the isolation of which was reported earlier in a brief note (*7*).

COMPARISON WITH DIPLANETIC DEVELOPMENT IN RELATED FUNGI

Although diplanetism has been observed in additional representatives of Phytophthora, no features associated with the phenomenon have come to light which are not amply illustrated in the dozen forms mentioned. If the papillate pink-rot fungus be assigned to *P. parasitica*, which, in spite of certain differences, it would seem to resemble more closely than any other of the better established species, and the fungi from Idaho potato tubers, Utah sugar-beet roots, and California tomato fruits be regarded as conspecific, the assortment of forms would be resolved presumably into about eight species. In all of the forms considered, the fashioning of the secondary zoospore took place directly within the cyst envelope or within the miniature sporangium, accompanied by noticeable writhing movements with retraction of protoplasm here and there from the confining wall, especially at the papilla or evacuation tube. The biciliate body after its passage through the open protuberance immediately swam away, except, as has been mentioned, in a few instances where a cilium adhered to the inner surface of the cyst wall. The manner of dis-

charge was therefore essentially the same as that generally characteristic of the ordinary sporangia produced throughout the genus.

The evident parallelism between the primary and the secondary swimming stages with respect to the fashioning and discharge of the motile zoospores in species of Phytophthora suggests analogous development in the genus Pythium. In discussing the germination of the zoospores of his *Pythium dictyosporum*, a species with filamentous sporangia parasitic on *Spirogyra insignis* Hass, Raciborski (*19, p. 284*) stated that if the germ tubes do not reach the Spirogyra filament, a new, small sporangium, which contains only one zoospore, is formed at the tip of the germ tube.

The small sporangia to which reference is made correspond apparently to the miniature germ sporangia produced by most of the Phytophthora forms discussed here. No details concerning the escape of the secondary zoospores were given, nor is it evident that the manner of dehiscence was observed.

Butler (*2*), however, gave a more satisfactory illustrated account of the diplanetic development manifested by his *Pythium diacarpum*. His description is confirmed by the writer's own observations on diplanetism in a number of species of Pythium, including, for example, *P. butleri* Sub., the parasite often associated with decay of various commercial cucurbitaceous fruits and of snap beans (*Phaseolus vulgaris* L.) in transit and on the market. The somewhat large primary zoospore of this fungus, after encystment measuring mostly 10 or 11μ in diameter, produces an evacuation tube usually 1.9 to 2.2μ in diameter and 3 to 25μ in length, the protoplasmic contents in the meantime displaying a vacuole of increasing size. Finally the refringent tip of the evacuation tube yields and the contents flow out to collect at the orifice, the streaming requiring usually from 30 to 45 seconds. The discharged protoplast soon begins to exhibit writhing movements, which, as cilia make their appearance and a grooved reniform shape is assumed, become increasingly energetic though restricted within a confined space. Usually about 20 minutes after the cessation of streaming the now violently active zoospore dashes away as a free-swimming body. The restriction in amplitude of movement of the secondary spore previous to its ultimate liberation is interpreted as evidence of the presence of a confining vesicle. Direct though somewhat dubious visible evidence of such a vesicle is provided now and then in indications of an approximately circular contour coinciding with the limits of the field of movement and never more than faintly discernible even in proximity to the mouth of the evacuation tube. Since in preparations of the same fungus the vesicles associated with the smallest sporangia of vegetative origin, producing 2, 3, or 4 zoospores, are often nearly if not quite as difficult to make out, the parallelism between the production of primary and of secondary zoospores is, indeed, little less than complete. While *Pythium butleri* represents a species with lobulate sporangia, similar diplanetic development was observed in extraordinary abundance in a congeneric form with undifferentiated filamentous sporangia isolated from diseased roots of sugarcane (*Saccharum officinarum* L.).

Cornu (*4*) mentioned *Pythium proliferum* De Bary and its varieties as examples of saprolegniaceous forms, the zoospores of which may emit zoospores similar to themselves as an alternative to putting forth vegetative germ tubes. The details of the repetitional develop-

ment were not discussed further. Cornu described development of zoospores in *P. proliferum* as taking place often within a vesicle formed at the extremity of the sporangium and at other times within the sporangium itself, the ready-fashioned zoospores in the latter case escaping directly when discharge occurred, since, although a vesicle appeared, it persisted only a few instants. The departure from the course of development generally prevalent in the genus Pythium is interesting when considered in connection with Butler's statement that in some cases in *P. diacarpum* much of the sporangial contents becomes divided at the mouth of an urn-shaped modification in the absence of a vesicle.

A more striking parallel with the condition set forth by Cornu is provided in the literature dealing with *Pythiomorpha gonapodioides* Petersen. According to the accounts of Petersen (*18*), Minden (*15*), and Kanouse (*14*), the fashioning and discharge of zoospores in the latter fungus would seem to be similar to that among species of Phytophthora. The vesicle described by Minden is evidently an ephemeral, poorly developed structure, bursting at an early stage in the evacuation of the sporangium to allow the completely formed zoospores to swim away without much delay—not differing greatly, therefore, from the bladderlike membrane noted by Dastur (*5*) in *Phytophthora parasitica*, and by Rosenbaum (*21*) in *P. cactorum* and *P. arecae* (Colem.) Pethyb. In Kanouse's account a second swarming was mentioned, the protoplasm passing slowly out of the cyst wall, though cilia were not observed, and no activity of the liberated naked cell other than the emission of a germ tube was represented in the accompanying illustration.

Because of the dearth of detail concerning the development of locomotive organs, it is not possible to refer the diplanetic development in question either to the type prevailing in the genus Phytophthora or in Pythium. In any case the mere presence of such development can hardly be advanced as a strong argument in favor of the distinctiveness of any form or group from either of these genera.

Although a fungus received in artificial culture from the Centraalbureau voor Schimmelcultures as having been contributed originally by Kanouse under the name *Pythiomorpha gonapodioides* has yielded no structures relating to diplanetism in the few irrigated preparations examined by the writer, a number of undoubtedly closely related species have revealed such structures in moderate abundance. Of these species, one isolated from diseased rootlets of ragweed (*Ambrosia trifida* L.) and referred to in a brief abstract (*8*) manifestly gives rise to the secondary swimming stage, not only by the fashioning of the motile body within the cyst wall and its direct delivery through an evacuation tube, but also indirectly by the production of miniature sporangia. The former mode of development is shown by the presence of empty spherical cyst envelopes usually measuring 11 to 16μ in diameter, each provided with an open evacuation tube 3 to 6μ in diameter and 5 to 15μ in length. When miniature sporangia are produced they are borne on germ sporangiophores mostly 2μ in diameter and ranging from 40 to 285μ in length. Frequently an empty cyst envelope with an open evacuation tube is provided with one or two narrower filamentous outgrowths suggesting abortive germ sporangiophores. Although unfortunately the fashioning and liberation of the secondary motile

bodies were not observed, the occurrence of two types of development paralleling those observed in various species of Phytophthora is not without interest.

However little tenable ground the writings on Pythiomorpha offer for the maintenance of that genus as a separate taxonomic entity, the fungus discussed by Kanouse as *P. gonapodioides* does not seem referable to Phytophthora. In the hands of the present writer the presumably identical organism never produced zoospores within the sporangium to discharge them full fledged through an orifice provided by a uniformly sessile papilla. On the contrary, development took place as in typical representatives of Pythium, by the discharge of undifferentiated contents into a vesicle in which division into zoospores was accomplished in 15 or 20 minutes. The papilla of dehiscence was sometimes sessile and at other times surmounted an evacuation tube 5μ or more in length. In versatility of asexual reproduction the fungus thus recalls Cornu's early description of similar behavior in *Pythium proliferum*. A larger measure of distinctiveness is embodied apparently in the somewhat unusual shape of the antheridium and its manner of application to the oogonium. In Kanouse's drawing of the sexual apparatus, the male organ is represented as an elongated clasping structure evidently applied lengthwise to the oogonium. A similar shape and manner of application is shown in Dissmann's figures (*6, figs. 19 and 33*) of the antheridium of his *Pythium proliferum*. As far as the writer is aware, no antheridum of similar type and of similar disposition with reference to the female structure has been recorded for any fungus definitely assigned to Phytophthora.

SUMMARY

In many species of Phytophthora, the zoospores after encystment give rise to secondary swimming spores similar to themselves except for a slight inferiority in size. The repetitional development follows either one of two courses. A papilla or tube of dehiscence, usually relatively broad and of variable length, may be produced, which on yielding at the apex permits the immediate escape of the completely formed secondary zoospore fashioned in the meantime from the protoplast with accompanying writhing movements visible in retractions here and there from the cyst wall. Or a slender but often rather long germ sporangiophore, terminating in a miniature papillate sporangium delimited by a basal septum, may be put forth, either from the periphery of the cyst or from an unsuccessful evacuation tube. The contents of the miniature sporangium are eventually discharged from the papillary orifice as a completely developed motile zoospore.

Only development of the more direct type was observed in *Phytophthora fagi* and in three unnamed congeneric fungi with nonpapillate, proliferous sporangia, one originating from a diseased Idaho potato tuber, one from a mature sugar-beet root in Utah, and the other from a decaying tomato fruit in California. In *P. hibernalis* only the second indirect type of repetitional development was noted. Both types were found in all strains referable to *P. parasitica* in a somewhat broad sense, including *P. parasitica* var. *rhei* and possibly a papillate form isolated from decaying potato tubers. *P. cactorum*, *P. citrophthora*, *P. melongenae* and a fungus

previously reported as having been isolated from a diseased Honeydew melon represent other congeneric parasites exhibiting direct as well as indirect diplanetism.

When dehiscence of the ordinary sporangia is partly or wholly frustrated, the imprisoned zoospores after encystment often give rise to a free-swimming stage through repetitional development. The germ sporangiophores perforating the sporangial wall or passing out through the papillary orifice to bear the miniature sporangia externally often lend a distinctive bristling aspect to the resulting reproductive arrangement.

Diplanetism was observed in various species of Pythium with filaments and with lobulate sporangia, as, for example, *P. butleri*, the encysted structure discharging its undifferentiated contents through a slender evacuation tube into a small vesicle where they are fashioned into a single motile spore. In Pythium as in Phytophthora, therefore, repetitional development follows the course generally characteristic of zoospore formation in the genus. In certain allied forms distinguished by elongated clasping antheridia closely applied lengthwise to the oogonium, and by mostly terminal and often proliferous sporangia, the occurrence of empty cyst envelopes and of germ sporangiophores terminating in miniature sporangia provide proof of the prevalence of diplanetic development.

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EFFECTS OF DEFOLIATION AND ROOT PRUNING ON THE CHEMICAL COMPOSITION OF SWEET-CORN KERNELS[1]

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INTRODUCTION

Since sweet corn in the fresh and in the canned condition is widely used for human food, it is desirable to know as much as possible of the relation of cultural practices and crop hazards to its composition and quality. In the field the corn plant is often mutilated in various ways; the root system may be injured by the plow or the cultivator; windstorms may throw the plants to the ground; hail may tear the leaves and greatly interfere with their normal functions; and insects may consume more or less of the foliage and injure the stalks. Theoretically, these conditions which directly affect the nutrition and development of the corn plant should affect the composition and quality of the grains. The object of the present work was to determine whether or not such is the case.

LITERATURE

Heckel (*7, 8, 9, 10*),[2] in a series of studies on the effect of emasculation on the sugar content of corn and sorghum, found that in general this treatment resulted at first in an increase in the amount of saccharose, levulose, and dextrose over that of the check plants, followed by a decrease in these sugars. Some variation was noted, however, in the behavior of different varieties. This worker made a study of the possible cumulative effect of traumatism on the sugar content in the progeny of corn detasseled for four successive years. An increase in total saccharose and glucose over that in the control plots was noted, the greatest percentage total increase being obtained from strains previously showing only moderate sweetness and the maximum being attained in 24 days after detasseling. No data were given on the chemical composition of the kernels in any of the work above cited.

Haller and Magness (*6*) investigated the relation of leaf area to the growth and composition of apples, and their work is mentioned here as indicating the importance of the amount of foliage in determining the nature of the crop. Apples grown with a large leaf area were higher in dry weight, sugar, and acids than those with smaller leaf surface, and the quality of the fruit was superior. Ripening took place more promptly.

Dungan (*4*) reported on the influence of plant injury and root-rot diseases on the physical and chemical composition of corn grains. In one series of experiments the stalks and shanks of ears were broken but not severed when the grain was in the milk stage and in another series when the grain was in the soft-dough stage. In other experiments the corn seeds were inoculated with root-rot organisms, and the

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[2] Reference is made by number (italic) to "Literature cited," p. 582.

effect on the composition of the grain of plants derived from this seed was determined. The effect of breaking the stalks and shanks of ears was the same on the yield and composition of the grain as premature harvesting. Breaking the shanks when the ears were in the soft-dough stage caused the greatest chaffiness in the grain. Chemical analyses showed no definite correlation between the apparent starchiness of the kernel and the actual quantity of starch. The percentage of total nitrogen, hemicellulose, and nonhydrolyzable material was distinctly higher in grain from ears produced on broken shanks than in grain from ears produced on sound shanks. The mutilation of the shanks resulted in a lower ether extract and starch content in the grain. Inoculation of the seed at planting time with root-rot organisms resulted in grain of a lower specific gravity than that from the uninoculated seed but no significant differences were noted in the chemical composition of the grain produced. There was slightly more nitrogen in the corn from inoculated seed and a little less ether extract and total sugar.

METHOD OF INVESTIGATION

The variety of corn used in the present investigation was Stowell Evergreen, grown at the Arlington Experiment Farm, Rosslyn, Va., during the season of 1927. The soil was a deep, fertile loam, and the seeding was heavy. When the plants were 8 to 12 inches high they were thinned, vigorous plants being left at 14 to 18 inches in the row. The rows were 3.3 feet apart. The plants used in the investigation were from two plots, one of which was planted on June 22 and the other on June 27. The combined plots, which were contiguous, totaled one-fifth of an acre. They were inspected daily from August 19 to September 10, and, for later identification, the newly forming ears were tagged on the day of the first appearance of silks, as in earlier studies (*2*, *3*, *11*). The plants on which the present report is based, between 400 and 500 in number, silked during the period from August 26 to August 31. They were treated as 10 separate groups, though the individual plants of each group were uniformly distributed within the two plots. The groups were treated as follows:

Group 1.—The leaf at the ear was left and the alternate leaves, both above and below the ear were severed close to the stalk. This was done on the day of silking.

Group 2.—All leaves except the one at the ear were removed on the date of silking.

Group 3.—All leaves were removed five days after silking.

Group 4.—Treated in the same manner as group 3, except that defoliation was performed 10 days after silking.

Group 5.—The leaves were all removed 15 days after silking.

Group 6.—The leaves were all removed 20 days after silking.

Group 7.—The plants were assumed to stand in the center of a square 6 inches on each side, and by means of a sharp spade the roots were cut to a depth of 18 inches on two adjacent sides of the square. It was assumed that one-half the roots were pruned away by this treatment. This was done on the date of silking.

Group 8.—The roots were pruned away to a depth of 18 inches on all four sides of the 6-inch square on the day of silking.

Group 9.—Partial defoliation and partial destruction of roots were performed, half the leaves and half the roots being destroyed on the day of silking.

Group 10.—Entirely normal, representative plants which received the usual cultivation and were not mutilated in any way served as controls.

Pressure of other work prevented a further elaboration of the root-pruning experiments.

None of these plants produced suckers or showed regeneration of leaves following defoliation.

At intervals of 5 days up to 30 days after mutilation, representative samples of 8 to 12 ears were collected where feasible from each group. The ears were taken to the laboratory, husked and silked and the kernels cut from the ear with a sharp knife, close to the cob. The material thus obtained was then thoroughly mixed and duplicate samples of 100 gm. each weighed out, covered at once with 95 per cent alcohol, and heated to boiling to inactivate the enzymes and prevent chemical changes. At the end of the season these samples were analyzed for carbohydrates, following the method of the Association of Official Agricultural Chemists (*1*).

During the period of the experiment the weather conditions were very favorable for corn. There was a sufficiency of rain, with normal summer temperatures and an absence of heavy winds or violent storms that might have injured the plants or interfered with the progress of the experiments.

The size of the ears and the yields of corn were not carefully determined, but a fair estimate was made by comparing the ears and yields of the mutilated plants with those from the normal controls.

EXPERIMENTAL RESULTS

The chemical data obtained from the analysis of samples from the different groups of plants are presented in Table 1, and a statistical analysis of the differences in total solids, total sugars, and acid-hydrolyzable substances between the various test samples and the controls is shown in Table 2. Use here was made of the symbols and formulas employed by Fisher (*5*) in testing the significance of the mean of small samples. In the headings of the various columns, $\bar{y}$ represents the mean of the small sample, s the standard deviation of this sample, and $\frac{P}{2}$ the probability of the value t derived by use of the formula $t=\frac{\bar{y}\sqrt{n'}}{s}$, or similar formula suited to the treatment of allied data, being exceeded in a positive or negative direction only, in other samples from the same universe. In the use of this symbol, only those values of $\frac{P}{2}$ less than 0.050 are considered to indicate significant differences; the smaller the value of $\frac{P}{2}$ the more significant the difference. A brief consideration of the formulas used in the present calculations is to be found in the footnotes to Table 2. The minus and plus signs in the $\bar{y}$ columns show respectively whether the mean of the total solids, total sugar, and acid-hydrolyzable substances of the test corn at different stages of development was less or greater than the mean of the control corn sampled at like stages of development.

TABLE 1.—*Effect of defoliation and root pruning on the chemical composition of the developing grains of Stowell Evergreen sweet corn*

Group No.	Treatment of plants	Age of ears from date of silking	Total solids	Alcoholic extract	Residue	Total sugar	Reducing sugar	Sucrose	Acid-hydrolyzable substances
		Days	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Half of leaves removed at time of silking	10	9.67	6.13	3.54	4.24	2.94	1.30	1.45
		15	13.83	8.02	5.81	5.78	2.30	3.48	3.25
		20	18.05	7.62	10.43	4.87	1.49	3.38	8.12
		25	22.46	7.40	15.06	3.98	.96	3.02	12.01
		30	25.97	6.36	19.61	3.43	.62	2.81	16.08
2	Leaves all removed except one at time of silking	10	8.87	5.60	3.27	3.92	2.15	1.77	1.30
		15	11.60	6.60	5.00	3.92	2.09	1.83	2.75
		20	17.79	7.24	10.55	5.16	1.00	4.16	5.80
		25	22.37	7.01	15.36	4.83	.87	3.96	12.60
		30	23.08	6.05	17.03	4.47	.78	3.69	14.13
3	Leaves all removed 5 days after silking	10	9.25	5.84	3.41	3.31	2.50	.81	.79
		15							
		20	14.21	6.48	7.73	4.36	1.28	3.08	3.86
		25	22.37	6.81	15.56	4.02	.83	3.19	10.26
		30							
4	Leaves all removed 10 days after silking	10							
		15	11.80	7.41	4.39	5.02	2.00	3.02	2.56
		20	15.46	6.76	8.70	4.64	1.23	3.41	5.22
		[1] 25	22.90	6.82	16.08	3.70	.75	2.95	11.90
		[2] 25	18.45	5.29	13.16	1.93	.79	1.14	7.96
		[1] 30	26.78	6.40	20.38	3.01	.56	2.45	14.67
		[2] 30	19.45	4.84	14.61	1.79	.55	1.24	9.01
5	Leaves all removed 15 days after silking	10							
		15							
		20	14.46	6.20	8.26	3.33	1.04	2.29	5.04
		25	21.63	5.48	16.15	2.04	.46	1.58	11.62
		30	25.34	4.91	20.43	1.84	.39	1.45	14.71
6	Leaves all removed 20 days after silking	30	26.92	4.16	22.76	1.88	.47	1.41	16.61
7	Roots pruned on 2 sides at time of silking	10	11.12	7.12	4.00	4.16	2.20	1.96	1.64
		15	15.88	8.56	7.32	5.76	2.07	3.69	4.03
		20	20.52	8.18	12.34	5.04	1.29	3.75	8.74
		25	24.46	7.08	17.38	4.01	1.01	3.00	14.25
		30	27.31	6.54	20.77	3.63	.44	3.19	17.23
8	Roots pruned on 4 sides at time of silking	10							
		15	15.78	8.36	7.42	5.73	1.74	3.99	4.08
		20	20.70	8.04	12.66	5.02	1.17	3.85	8.99
		25	27.70	7.84	19.86	4.46	.76	3.70	16.28
		30	31.30	5.88	25.42	3.23	.71	2.52	21.1
9	Half of leaves removed and half of roots cut at time of silking	10	9.63	6.06	3.57	3.25	1.83	1.42	1.43
		15	14.70	8.02	6.68	4.90	1.90	3.00	3.61
		20	18.86	7.80	11.06	4.94	1.01	3.93	7.85
		25							
		30	29.45	6.14	23.31	3.02	.58	2.44	19.34
10	No treatment (used as control)	10	9.96	6.00	3.96	4.06	2.88	1.18	1.61
		15	14.44	7.60	6.84	5.76	1.84	3.92	3.76
		20	18.82	7.16	11.66	4.84	1.36	3.48	8.35
		25	23.17	6.72	16.45	4.17	.74	3.43	12.78
		30	25.45	5.94	19.51	3.37	.62	2.75	16.19

[1] Normal grains. [2] Shriveled grains.

TABLE 2.—*Significance of differences in total solids, total sugars, and acid-hydrolyzable substances between corn from defoliated and root-pruned plants and that from untreated controls, as determined by statistical analysis*

[Calculated from the data of Table 1]

Group No.	Total solids			Total sugars			Acid-hydrolyzable substances		
	$\bar{y}$	s	$\frac{P}{2}$	$\bar{y}$	s	$\frac{P}{2}$	$\bar{y}$	s	$\frac{P}{2}$
1	−0.372	0.531	0.097	+0.020	0.134	0.378	−0.356	0.278	0.024
2	−1.626	.915	.009	+.020	1.134	(1)	−1.222	1.052	.032
3	−2.040	2.226	.130	−.460	.300	.063	−2.610	1.836	.072
4	[2] −1.235	2.160	.169	−.443	.227	.017	−1.683	.999	.023
5	−2.003	[3] .564	([4a])	−1.723	.352	.007	−1.983	1.160	.049
6									
7	+1.490	.288	([5])	+.080	.167	.174	+.640	[3] .362	([4a])
8	+3.400	[4] .624	([4b])	+.075	1.953	(1)	+2.362	[5] .728	([4d])
9	+.990	2.019	.199	−.480	.449	.085	+.580	1.720	.275

[1] More than 0.9.
[2] The data given under 4 are based on the results from normal-appearing grains.
[3] In these cases a trend with age was shown, and the data here were calculated from the line of regression. The formulas used were:

$$s=\sqrt{\frac{S(y-Y)^2}{n'-2}} \text{ and } n=n'-2,$$

in which $(y-Y)$ = the deviation of y from the calculated value Y on the line of regression.
a = mean of the y's.
n' = number of items in the small sample.

In all other cases the formulas

$$s^1=\frac{S(y-\bar{y})^2}{n'-1},\ t=\frac{\bar{y}\sqrt{n'}}{s},\ \text{and } n=n'-1 \text{ were used.}$$

[4] In these cases it is not possible to express the significance of the difference between test and control by a single value. (Fisher, 6, *pp. 99–123.*) The formulas for the lines of regression are:
(*a*) $y=12.628-0.425\,x$, the range of the x's being 20 to 30 days, inclusive.
(*b*) $y=3.881+0.324\,x$, the range of the x's being 15 to 30 days, inclusive.
(*c*) $y=-.648+0.064\,x$, the range of the x's being 10 to 30 days, inclusive.
(*d*) $y=-5.229+0.337\,x$, the range of the x's being 15 to 30 days, inclusive. In all cases the interval of observation was 5 days.
[5] Less than 0.1.

EFFECT OF PARTIAL AND COMPLETE DEFOLIATION

On the whole, partial defoliation at the time of silking (group 1) made but little difference in the chemical composition of the grain as compared with that from normal plants, though the acid-hydrolyzable substances were definitely lower than in the control corn. In the case of the corn from plants entirely defoliated at the time of silking (group 2) the total solids and the acid-hydrolyzable substances were significantly lower than in the control corn.

The most marked effect of defoliation at this stage of development, as determined by careful observation, was in the size of the ears and in the yields. In the case of the plants from which half of the leaves were removed the ears were only one-half to two-thirds as large as normal ears, and on the ears of the nearly completely defoliated plants the kernels were smaller in size than on normal ears. They appeared to have developed more slowly, but in all other respects seemed normal. There was considerable variation in the behavior of the plants treated in this way at the time of silking. Some produced no seed at all, the ovules failing to develop, while others developed from 50 to 200 grains. The normal plants produced from 350 to 400 kernels to the ear.

The data showing the effect of complete defoliation five days after silking on the chemical composition of the corn (group 3) are incomplete, but so far as they are available they indicate results similar to those where the leaves were removed at the time of silking. The differences between this corn and the controls, however, were less significant, though more complete data might have shown more marked effects of this treatment. Inspection of the ears tof his group showed that some kernels started to develop and subsequently failed. They had developed sufficiently, however, to be included in the sample, and although they formed but a relatively small percentage of the total sample, their effect on the composition was perceptible.

Defoliation 10 days after silking (group 4) resulted in marked physical and chemical differences in the grains. The ears from this defoliated corn sampled 15 and 20 days after silking showed some kernels that seemed to be developing normally and others that had failed completely to develop. In the corn sampled 25 and 30 days after silking the differences were so marked that two sets of samples were taken, one of the normal-appearing kernels and another of the shriveled grains. Differences in the chemical composition of these two sets of samples are shown in Table 1. The "normal" kernels had approximately the same composition as those from the plants defoliated at the time of silking. The sugar and the acid-hydrolyzable substances were significantly lower than in the kernels of the control corn. In the shriveled grains, on the other hand, the total solids and the sugar and acid-hydrolyzable substances were much lower than in the control samples. It is apparent that the incoming storage material, in the case of the ears of this lot, was insufficient to meet all needs, and a part of the kernels received it all, the remaining being left to wither.

Cessation of development did not occur at exactly the same time in all kernels that failed to mature properly; consequently, in the later stages of development the ears showed fewer normal grains than those harvested earlier. There was, of course, considerable variation in the behavior of individual plants. A small percentage of ears was found on which all the kernels appeared to have stopped developing at the same time. In such cases the plant as a whole ceased to function and usually withered and died prematurely.

The corn from plants defoliated 15 days after silking and sampled after 20, 25, and 30 days (group 5) was characterized by a significantly lower content of sugar and acid-hydrolyzable substances than the control corn.

The single sample from corn defoliated 20 days after silking and sampled 30 days later (group 6) showed higher total solids, lower sugar content, and slightly higher content of acid-hydrolyzable substances than the corn from the control plants. It is probable that these differences were due, in part at least, to the drying out of the corn prior to sampling. The data are too few, however, to warrant definite conclusions on this point.

The ears of this lot showed again the unequal development of the grains. Differences in development could not be discerned as soon after defoliation, however, as in the corn mutilated at or shortly after the silking time. There was also a larger percentage of ears on which grains failed to continue development.

EFFECT OF ROOT PRUNING

The total solids and the acid-hydrolyzable substances of the corn from which half the roots were removed (group 7) were significantly higher than those of the control corn. The differences in total sugar content were not significant. The removal of the roots from the four sides of the 6-inch square (group 8) yielded effects of the same nature, but the figures were more striking. The outstanding results of root pruning were a higher content of total solids and acid-hydrolyzable substances than that of the control corn, whereas lower total solids and a lower content of acid-hydrolyzable substances characterized the defoliated corn. The signs of the data in the $\bar{y}$ columns of Table 2 indicate this clearly.

The removal of half the roots and half the leaves from the same plants (group 9) gave analytical results similar to those of group 8, but the size of the ears in this case was much reduced. The differences between the corn from these plants and that from the controls were not significant.

As indicated by the size of ears, reduction in yields of the root-pruned corn was marked, particularly in the case of those plants which were more severely treated; but the reduction was not so great as that resulting from defoliation.

GENERAL DISCUSSION

In considering the foregoing data it must be borne in mind that the nature and degree of the response of the corn plants to the different treatments was not, from the standpoint of the individual plants, always constant or consistent. Considerable variation in the behavior of individual plants was encountered. So far as the conditions of the experiment permitted, however, the data presented represent the group response.

In these experiments the effects of mutilation of the plant on the chemical composition of the grain were most clearly exhibited in those lots treated 10 and 15 days after silking. In the case of these plants mutilated when the kernels were in an earlier stage of development, the kernels that later failed to grow withered away before significant size was attained, and hence had no significant effect on the character of the sample. Those plants mutilated when the grains were more than 15 days of age yielded corn more closely approaching normal grain according as the treatment was longer and longer delayed.

In considering these data the climatic conditions under which the corn was grown should be held in mind. During the course of the experiments temperature and moisture conditions were very favorable for the growth and maturing of the corn. If hot winds had prevailed at any period subsequent to mutilation, the failure of kernels resulting from root pruning at least would have been greater, and higher total solids of the grain would doubtless have resulted in the case of the defoliated plants.

Yields, as shown by the size of the ears and the number of kernels, were most severely affected when the plants were defoliated at the time of silking. Partial defoliation was found to be much more disastrous to yields than partial root pruning.

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In an earlier publication (*3*) it has been shown that quality in sweet corn is dependent on tenderness of kernel hull, the nature of the polysaccharides present, the sugar content, and other factors. Any condition exerting an unfavorable influence on the uniform composition and quality of the properly harvested corn therefore must of necessity be taken into careful consideration. From these experiments it would appear that, from the standpoint of quality of the canned product, hailstorms or other agencies destroying the foliage would be most severely felt if they occurred from 10 days to 2 weeks after the corn had flowered. The same would probably be true of drought or other unfavorable seasonal conditions.

SUMMARY

Partial or nearly complete defoliation of corn plants at the time of silking caused only small differences in the chemical composition of the developing grains. It decreased the total solids somewhat and seemed to slow down slightly the rate at which the grains matured. Root pruning done at this time resulted in corn of high total solids, and the rate of maturing seemed to be slightly increased. Mutilations at this time tremendously decreased yields, as shown by the small size of the ears and the small number of kernels. Under the conditions of these experiments defoliation was more disastrous than root pruning.

Defoliation performed after development of the kernels was well underway resulted in a very marked change in the chemical composition of the grain. The effects were most severely felt when the plants were defoliated 10 and 15 days after silking, under the climatic conditions prevailing during the period of the experiment. Yields, as indicated by the size of ears and the number of kernels, were also severely reduced under these conditions, but less severely than when defoliation was performed at the time of silking. Beyond the 15-day stage the undesirable consequences of defoliation were progressively lessened as the treatment was delayed.

When the corn plant was mutilated there was a marked difference in the behavior of the grains. Some kernels developed normally, while others on the same ear entirely failed to develop. It appeared that when the organic nutrients were insufficient for all the supply was unequally distributed, a few grains receiving all.

From a consideration of the analytical data it is concluded that any condition affecting the vegetable development of the plant adversely would affect the quality of the canned product most unfavorably if it became operative 10 to 15 days after the silking of the corn.

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